SHORT ROTATION CULTURE OF WILLOW CLONES ACROSS CANADA: GROWTH REQUIREMENTS AND IMPLICATIONS FOR SOIL NUTRIENTS AND GREENHOUSE GAS BALANCES

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ABSTRACT

The cultivation of willow (*Salix* spp.) is being investigated as a potential feedstock for biomass energy in the Canadian prairies. For this purpose, and despite willow's high nutrient and water demand, high rates of productivity can ideally be achieved and maintained while minimizing inputs and greenhouse gas (GHG) emissions. Therefore, plantations of *Salix purpurea* 'Hotel' grown at seven sites across Canada—encompassing a large climate gradient and a variety of soils—provided an opportunity to assess the impacts of site quality on biomass production. Soil and climate variables as well as internal measures of nutrition and water stress were correlated with harvested biomass via linear, non-linear and multiple linear regression. Soil at nine sites were compared to reference sites in order to examine the effects of willow plantation establishment on soil nutrient pools and GHG efflux. The effects of initial nutrient concentration and growth rates on change were also examined. Greenhouse gas efflux (CO_2, CH_4, N_2O) was directly measured using the vented chamber method at two of the sites. Lastly, a new method for measuring standing biomass via ground based digital imaging was developed and validated.

Total elemental composition of soil, in particular the presence of Ca, was found to have the greatest correlation with willow productivity ($r = 0.89$, $P < 0.01$). Surprisingly, precipitation was not the dominant control for the majority of the sites. Nutritionally, N ($r = 0.44$, $P < 0.01$ total soil N) and K ($r = 0.52$, $P < 0.01$ foliar K) were dominant for increasing willow productivity although both N ($P < 0.05$) and K ($P < 0.10$) were depleted from the previous land use with willow plantation establishment. This indicates increasing nutrient deficiencies of N and K may be become problematic in future rotations for maintaining productivity. Carbon was consistently diminished in the upper 20 cm of soil ($P < 0.05$) regardless of previous conditions or productivity although previous land use is a contributing factor with less depletion on cultivated soils. However, soil CO_2 emissions were greatest under larger trees indicating increased C cycling with increasing productivity. Seasonal differences in N_2O emissions and CH_4 consumption from traditional agriculture and mature tree stands were not detected.

The calcareous soils common in the Canadian prairies provide opportunity for willow production, though fertilization with N and K may be required to sustain adequate growth.

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1. INTRODUCTION

With growing concerns over global warming, the interest in alternative renewable carbon (C) responsible energy continues to rise. Of the options for renewable energy, biomass energy fits well with current energy use patterns due to its ability to be processed into a variety of useable forms (Volk et al., 2004). Ethanol, derived through fermentation of biomass, can provide a liquid fuel suitable for automotive use. Biogas [a mixture of methane (CH_4) and carbon dioxide (CO_2)], also from biomass, can supply energy in much the same way as natural gas. Finally, biomass itself can be burned independently or for existing power plants co-fired with coal for the production of electricity. One potential source for biomass fuel is the willow tree. Willow (*Salix* spp.), grown under short-rotation intensive culture (SRIC), could provide some of the biomass for all of these energy products (Abrahamson et al., 2002; Volk et al., 2004). Willow can be harvested every three or four years. After harvesting, it regrows vegetatively without the need for replanting for seven or possibly more rotations (Abrahamson et al., 2002). Once harvested, the willow can be used directly or processed for ethanol or biogas. Thus, biomass derived from willow can potentially offset some of the greenhouse gas (GHG) emissions from fossil fuels (Volk et al., 2004).

The Canadian prairies are a promising region for SRIC. They are generally characterized by fertile soils but low precipitation. The southern portions of the Canadian prairies can be very dry, thus supporting very little tree growth. However, towards the north the prairies undergo a transition where cooler temperatures and more precipitation allow stands of trees and eventually forests to persist (Richards and Fung, 1969). Some of the native willow species found in these regions are well suited to SRIC and breeding programs will only enhance their capabilities. Therefore, there is a good match between SRIC using fast growing willow and much of the Canadian prairies. Additionally, the human population in this area is dispersed among many small rural communities. SRIC could provide for a substantial portion of these communities energy needs by utilizing locally grown biomass.

Before willow can be confidently used as a sustainable energy source in the Canadian prairies, a number of questions must be answered. One of the biggest questions is, “Can willow

be grown at high productivity in Canada and can this growth be maintained?" Another major question revolves around GHG emissions, including CO_2, nitrous oxide (N_2O) and CH_4, from willow as SRIC. The net effect of replacing fossil fuels with willow based biomass fuels is yet to be fully understood including the potential for willow plantations to reduce the CO_2 already in the atmosphere by increasing C sequestration in soils.

Willow plantations previously established across Canada by the Canadian Forest Service were used to complete this research. Willow biomass trials were planted in 2005 and 2006 at several sites from Alberta to Quebec. Sites included a wide range of climatic and soil conditions. Each site included numerous willow clones although not all sites contained the same clones. *Salix purpurea* 'Hotel' was included in more plantations than any other clone due to availability of planting stock. It also proved to be relatively resistant to insects, disease, herbivory and cold compared to other clones established on the same sites. Therefore, this study made use of the nine sites from this network of plantations that included 'Hotel'.

This dissertation is written in the style of a collection of journal articles (Chapters 3-6) such that each chapter is comprised of one or two articles. The objectives of the chapters contained within this dissertation are as follows:

Chapter 1: provide a brief introduction into the context of willow as a bioenergy crop and some of the questions around establishing plantations in the Canadian prairies,

Chapter 2: provide a summary of the current state of research with respect to willow productivity, the effects of plantation establishment on soil nutrients and GHGs and techniques for monitoring productivity,

Chapter 3: test the hypothesis that productivity can be related to climate and soil variables so that, in the future, the best sites for plantations can be selected,

Chapter 4: test the hypothesis that willow plantation establishment will not alter soil nutrient and C storage with emphasis on highlighting potential concerns for maintaining productive sites,

Chapter 5: compare the effects of developing willow plantations on soil GHG efflux to that of traditional agriculture and permanent tree cover to test whether willow plantations can decrease soil GHG emissions,

Chapter 6: develop and test for accuracy and precision a non-destructive method of estimating standing biomass with the intent that it will aid in future monitoring of productivity in high density plantations for management and research purposes,

Chapter 7: synthesize the articles within this dissertation to provide conclusions based on this research as well as the need for future research in the area.

2. LITERATURE REVIEW

2.1 Willow as a Potential Bioenergy Feedstock.

Other plant derived sources of energy exist but willow grown in SRIC has many advantages (Volk et al., 2004). For example, some concerns have arisen with corn (*Z. mays*) based ethanol. Nitrogen (N) fertilizers used to boost productivity increase energy inputs and may contribute to the release of N_2O into the atmosphere which could offset substantial portions of the reductions in CO_2 emissions (Kim and Dale, 2005). As well, diverting corn into the energy sector could increase food scarcity as more corn is converted into ethanol for energy (Pimental, 2003). SRIC addresses these issues and offers an alternative source of biomass energy without many of the complications. In addition, willow offers a much higher energy return than many other biomass energy sources including corn (Volk et al., 2004) and also it has been shown that willow can be used effectively for environmental benefits such as erosion control, phytoremediation, riparian buffers and increased biodiversity (Khanna and Ulrich, 1984; Ledin and Willebrand, 1995; Mirck et al., 2005; Volk et al., 2004). However, the ability of biomass plantations to increase biodiversity are minimal compared to other treed systems that utilize longer rotations with greater heterogeneity; i.e., the necessity of weed control in SRIC, coupled with the short rotations makes for little understory development (Christian et al., 1997; Christian et al., 1998). Additionally interaction effects with the surrounding landscape will mean that willow plantations may reduce heterogeneity by infilling open fields in an otherwise treed landscape (Berg, 2002). Willow SRIC bioenergy may also have some environmental drawbacks, such as varying amounts of air pollution with direct combustion (Meyer, 2012). Life-cycle analysis has demonstrated that willow grown in SRICis a viable energy source (Berndes et al., 2001; Heller et al., 2003; Goglio and Owende, 2009). However, validation of certain aspects of nutrient cycling, site quality indexing and GHG efflux in willow plantations have been lacking for the Canadian prairies.

While biomass fuels emit CO_2 as they are combusted, it is necessarily balanced by the amount of CO_2 absorbed by photosynthesis during production. Because all of the C within the biomass is originally atmospheric, it is not possible to directly increase global CO_2 levels by

burning biomass fuels so long as the harvested biomass is regrown. This means that as biomass fuels replace fossil fuels there is a net reduction in CO_2 emissions. Although there is no increase in CO_2 emissions directly as a result of burning biomass fuels, processing and transportation using current infrastructure requires the use of fossil fuels (Volk et al., 2004). Ideally, transportation and processing could be done using biomass fuels but current infrastructures do not allow for this. Although some fossil fuel CO_2 is released into the atmosphere it may be possible to maintain that biomass energy is net zero C if more energy is produced than consumed. So long as biomass energy is used to offset more fossil fuel energy elsewhere than was used in its production and transportation, it is inconsequential to the C neutrality argument whether or not the biomass production and transportation uses fossil fuels. Therefore, it is the amount of energy and the energy input to output ratio that affects the C budget not the source of the energy. Should it be proven that willow biomass production sequesters C into long term storage in the soil we can even consider it net negative atmospheric C. However, if land-use change depletes soil C stocks, willow biomass energy would be a source of atmospheric CO_2. Therefore the effect of plantation establishment on soil GHG efflux requires examination. What is equally if not more important is the land base required to produce the energy and the energy input to output ratio. Addressing this issue requires identifying sites with potential for acceptably high rates of productivity with minimal need for energy intensive management. As well, the effects of SRIC willow on soil nutrients and C must also be known so that growth can be maintained with minimal amendments. Because GHG balances, plantation feasibility and long term effects of plantations are all tied to productivity, further work on developing methods of measuring standing biomass non-destructively are also needed.

2.2 Factors Affecting Site Quality for Willow

The viability of willow as an agroforestry crop depends on its yield. Willow grown as SRIC has little large scale potential as a specialty wood but success depends on producing high volumes of biomass. Maximizing productivity while minimizing inputs is essential for willow bioenergy to be a sustainable energy source. Good site selection is key to achieving this balance

such that amendments only be added in response to deficiencies rather than as a prescription for achieving high growth rates on unsuitable land (Mitchell et al., 1999).

Traditional forestry often characterizes site quality based upon previous rotations on the same or similar sites (Ung et al., 2001). However, willow is to be established as afforestation in monocultures, therefore, there are no pre-existing stands at which to develop site quality indices for willow plantations. Site quality must be estimated based on soil and climatic variables to estimate site potential (Carmean, 1996). In choosing the ideal sites for willow plantations, it is also important to consider economic and food production issues. The economics of biomass energy without some form of subsidization remain uncertain (Keoleian and Volk, 2005) and as such land which is highly productive for agriculture is not likely to widely be converted to SRIC. Willow has been shown to grow well on good agricultural land as well as land which has been deemed 'marginal' for agriculture (Abrahamson et al., 2002). Therefore, the best choice from an overall land management perspective is likely to grow willow on 'marginal' soils capable of supporting viable plantations without affecting food security or being economically impractical. Because this 'marginal' land is likely to be the land converted to SRIC, we must understand the exact soil to growth relationship of willow to make informed decisions about whether a soil is productive enough to support a viable plantation.

Factors that will influence site index and site quality include soil texture, soil nutrients and climate (Carmean, 1996; Ledin and Willebrand, 1995). At the broadest scale, climate variables are often the most important factors in determining productivity. Higher average temperatures, longer growing seasons and abundant water usually translate into higher productivity. Where water is abundant, willow production can be expected to rise compared to locations with water scarcity (Lindroth and Cienciala, 1996). At a smaller scale, topography can have impacts on production similar to climate. Topography influences run-off patterns as well as water holding capacity, thus affecting how much of the precipitation is actually available to trees (Dumanski et al., 1973). Aspect and steepness of a slope can also cause microclimate changes to temperature and moisture which can affect various trees differently (Chen et al., 2002). At local or microsite scales, the influence of soil texture, pH and nutrients become more apparent. For each tree species, there is an optimum pH and soil texture range (Carmean, 1996). Increased

nutrient availability generally suggests increased productivity but this is not always true: in some instances, increased nutrient availability can lead to increased competition or even toxic effects. In trembling aspen (*Populus tremuloides*), N, C, calcium (Ca), magnesium (Mg) and sulfur (S) have been shown to be positively correlated to growth (Chen et al., 1998; Coyne and Van Cleve, 1977; Paré et al., 2001). However, Chen et al. (1998) found a negative correlation between P and aspen growth. Parent material, which influences soil texture, nutrient availability and soil water (Birkeland, 1999) accounts for some of the variability in growth between sites (Carmean, 1996).

Willow is nutrient intensive as compared to other temperate tree genera (Cornelissen et al., 1997). Labrecque et al. (1998) demonstrated that fertilization of willow in Quebec induced a positive growth response and that N was the most limiting nutrient. Similarly, Adegbidi et al. (2003) found that fertilizing with either organic amendments or slow release inorganic N increased willow production. Long-term gains were highest with organic amendments of sewage sludge or manure suggesting that the improvements in soil structure, organic matter, pH, extractable phosphorus (P) and exchangeable cations associated with organic amendments also play a role in increasing productivity.

As of yet soil-site relationships for willow grown in SRIC plantations have not been determined in the Canadian prairies. In the north-eastern United States, Abrahamson et al. (2002) classified soil texture, structure, drainage, pH and depth into suitable and unsuitable for willow biomass production. They found that deep, moderately well drained soils with abundant water and nutrients and pH between 5.5 and 8.0 had the best willow growth. Sandy loams to clay loams produced the highest yields, but Abrahamson et al. (2002) also noted that clay was found to have a negative effect on early growth. In subsequent rotations, however, production in higher clay soils may be superior to low clay soils. Arevalo et al. (2007) demonstrated the importance of site for willow biomass production noting that site-specific information was necessary for the highest level of accuracy in estimating biomass. Therefore, there is a need to build on this knowledge and study the soil-site relationships in the Canadian prairies.

2.3 Effects of Plantation Establishment on Soil Nutrients

It is well known that trees have pronounced effects on the soil. Trees are able to access resources from deeper within the soil profile, enhance soil mineral weathering through the production of organic exudates, change hydrologic conditions to decrease leaching, store nutrients within their biomass, add nutrients to the soil through litterfall, alter pH and suppress competition (Attiwill and Adams, 1993; Augusto et al., 2002; Binkley and Giardina, 1998). As a result nutrient cycling in a forest can be very efficient (Binkley and Giardina, 1998).

Afforestation introduces trees onto land where previously trees did not exist and as with natural forests can have significant effects on nutrient pools and cycling (Ross et al., 1999). Due to high nutrient requirements (Mitchell et al., 1999) and export of nutrients with harvested biomass (Adegbidi et al., 2001; Augusto et al., 2002), nutrient cycling in SRIC is not expected to be as tight as in a forest, natural or otherwise. Adegbidi et al. (2001) calculated and compiled average nutrient removal rates for various locations and growth rates. They found that nutrient removal is sufficient to necessitate anthropogenic inputs. They did not however compare nutrient removal to changes in soil nutrient pools.

Biologically mediated processes may increase nutrient sequestration and availability; and consequently, rapid tree growth does not always occur at the expense of soil nutrients (Bélanger et al., 2004). Faster growth and high densities may lead to intensification of biologically mediated processes found in natural forests. It can also be expected that breeding trials will produce faster growing trees. Although nutrient use efficiency (more biomass produced with the same amount of nutrients) can be increased through breeding, large amounts of nutrients will continue to be taken out of the soil and this will likely increase with increased growth (Simon et al., 1990; Bormann et al., 1998, Adegbidi et al., 2001). Unless they are replaced by biologically mediated sources such as mineral weathering and decreased leaching, nutrient pools will likely dwindle and fertilization will be required.

Clearly, maintaining soil nutrient supply in willow plantations is not as simple as adding fertilizer to replace the exact quantity of nutrients exported with biomass. There have been many studies noting high nutrient use of willow (Simon et al., 1990; Kopinga and van den Burg, 1995; Cornelissen et al., 1997; Mitchell et al., 1999). However, the net effects of willow plantation on

soil nutrient stocks in the field in terms of potential of these sites to maintain productivity has been relatively unstudied, with no study on Canadian soils. Unfertilized willow plantations also must be examined so that potential need for amendments may be more efficiently predicted.

2.4 Assessing the Greenhouse Gas Impact of Plantations

Much of the intent behind willow grown as SRIC is as a biomass energy feedstock. While it is important to investigate the impact of all systems on GHG balances, the performance of willow with respect to its effect on GHGs will be critical in justifying its use. Under the current economic climate it is estimated that willow biomass energy will cost more money to produce than fossil fuels (Keoleian and Volk, 2005). Therefore, there needs to be reason for either subsidies at the government level or premium prices for the consumer. Unlike corn based ethanol, there is no established industry that will benefit from subsidized willow biomass production. Therefore any push for willow grown as SRIC will be made on environmental grounds. Willow can be used in phytoremediation applications, in particular wastewater treatment, and this has merit on its own (Aronsson and Perttu, 2001). However, the large scale adoption of willow depends upon its ability to positively impact GHG balances, thus prompting government incentives or premium prices and ultimately making willow production a financially attractive option. For a willow biomass industry to develop other economic and social factors would also need to be considered such as changes to the labour force and competition with other industries for natural, economic, and human resources.

Willow biomass has been found to be a viable source of energy in theoretical life-cycle analyse (Berndes et al., 2001; Heller et al., 2003; Goglio and Owende, 2009). Fossil fuel consumption required for the production of willow can be easily compensated for by offsetting fossil fuel use elsewhere. At the farm gate, the energy output to input ratio may be as high as 55:1 according to life cycle analyses, although at the actual end use ratios may be much lower (Matthews, 2001). However, this high of an energy ratio has yet to be operationally achieved. More reasonable estimates may be between 7:1-19:1 with large differences attributed to differences in fertilizer application, drying and transportation (Goglio and Owende, 2009). Nevertheless, this demonstrates the potential of willow biomass from an operational perspective.

Agriculture and land use change are known to be leading contributors to atmospheric GHGs [Intergovernmental Panel on Climate Change (IPCC), 2007]. While there is a general assumption that planting trees sequesters C this does not always apply, and there is little conclusive field data on the effects of the interactions between willow and soils on GHG budgets (Keoleian and Volk, 2005). The aboveground biomass of willow plantations is only a very temporary store as it will be returned to the atmosphere as CO_2 upon use as bioenergy. Therefore it is the below ground portion including both roots and soils that is a potential source or sink. If willow is planted as afforestation it is most likely to be planted on agricultural land that is C depleted. This presents a very large potential one time sink for C. However, land use conversion is typically accompanied by an initial loss in soil C followed by a move towards its C saturation point (Guo and Gifford, 2002). Willow plantations are new to Canada, in particular to the prairies, and as such their impact on soil C is unknown.

It is unclear whether intensively managed willow plantations will behave, in terms of GHG dynamics, more like agricultural systems, natural forests or some combination of the two. Continued disturbance prevents the accumulation of soil C and so there is question as to whether rotations and plantation life cycles are long enough to accrue soil C above pre-establishment levels in northern climates. Research in other plantations has shown that it may take >30 years for C gains in northern soils due to initial C debts from land use conversion (Laganière et al., 2010). Unfortunately, this is longer than the typical seven rotation life cycle of a willow plantation (Volk et al., 2004) and even with reestablishment of willow on the same site there will be intensified disturbances to soils associated with the termination of one willow crop and the initiation of another. Therefore, the effect of land use conversion must be measured and included in life cycle analyses. Productivity of willow plantations is however engineered to be as high as possible so that it would be expected that more soil C is cycling below ground and therefore more is being sequestered for the long-term. The effects of plantations on other major GHGs must also be assessed. There are currently no direct measures of N_2O and CH_4 fluxes from willow plantation soils to compare to agricultural soils and soils under permanent tree cover. Agriculture is the largest source of N_2O [Intergovernmental Panel on Climate Change (IPCC), 2007]; therefore, it is expected that converting land to willow will result in less N_2O emissions.

Additionally, forests typically have the highest rates of CH_4 oxidation (Le Mer and Roger, 2001). Thus willow plantations established on previous agricultural land are expected to be beneficial to mitigating atmospheric N_2O and CH_4, although this remains to be tested.

2.5 Monitoring productivity non-destructively

SRIC offers a certain amount of flexibility in the timing of harvest which is not possible with annual crops. Although rotations are designed to cycle through a set number of years (often three), it is not only possible but beneficial in certain circumstances to harvest earlier or later. For example, if due to excessive weeds or unfavorable growing conditions the trees have stunted production then it may be economically optimal to delay or accelerate harvest by a year or two. On the other hand, in the case of severe winter kill, it may be best to harvest the standing biomass and encourage the trees to start again from the bottom. In this way, whatever biomass exists is recovered and plant health is improved. The decision to harvest earlier or later requires knowledge of how much biomass is harvestable at a given time. Additionally, should C credits be developed, on site assessments of productivity will be required to determine the amount of sequestered C. Therefore there is a need for a method of rapid assessment of biomass within willow plantations.

The ideal method for biomass estimation is non-destructive, inexpensive, quick, independent of clonal variety, and accurate. Verwijst and Telenius (1999) reviewed the subject of biomass estimation in SRIC. At the time, destructive random sampling within a plantation and scaling results to represent the whole was found to be effective and accurate but deemed impractical. Thus there was need to borrow from techniques used in traditional forestry and rely on allometric relationships for developing methods of biomass estimation. However, converting these small measures to stand scales would prove problematic due to the complex canopy architecture of willow (Verwijst and Telenius, 1999). Nevertheless, until recently nearly all methods of biomass estimation in high density plantations have continued to rely on allometric relationships and have had difficulty in their ability to estimate biomass across sites (Arevalo et al., 2007).

Again traditional forestry offers some potential solutions to these problems. Many tools and techniques have been developed over time for measuring stand level forest structure. Leaf area index is one example of canopy structure that has been measured (Norman and Campbell, 1989). Photography is best known in forestry for characterizing canopy structure (Rich, 1990; Escobar et al., 2005) although the information contained in an image can be used for many more applications. Heisler and Dewalle (1988) used photographs to determine effectiveness of shelterbelts as windbreaks. Photographs have even been previously used for estimating biomass of standing trees. Ter-Mikaelian and Parker (2000) calculated the area, within a photograph, of a white pine (*Pinus strobus* L.) silhouette against a screen. This technique was able to predict biomass with strikingly high precision ($r^2 \leq 0.98$). Although the use of a screen is impractical within a high density plantation, the potential for a photographic technique for measuring standing willow biomass had been demonstrated. Advances in consumer digital cameras have both increased the ability and decreased the cost of digital image processing and analysis—two prerequisites for making the method as accessible to producers as possible.

More recently, there have been more attempts at indirect measurement of willow biomass using digital photography. As part of this dissertation, Ens et al. (2009) developed the optical stem density method for biomass estimation in willow plantations. Hangs et al. (2011b) used a theoretically very similar technique. Straatsma et al. (2008) compared a screened parallel photography technique to terrestrial laser scanning on natural forests (including willow) and found photography to be a superior and very effective method for determining stand density. Thus the concept has been proven and now needs validation for cross-site application.

Preface to Chapter 3

The success of a Canadian willow biomass industry requires thoughtful regional planning. Willow plantations need to be centered around processing facilities such that transportation of bulk biomass is minimized. Therefore, it is not enough to simply develop the geographic distribution of the industry by trial and error. Rather, it is essential that the productivity of willow species be predictable from measurable climatic and soil characteristics. In this chapter, the factors which dominate site quality for willow are identified and characterized. In doing this it becomes possible for future researchers and industry leaders to choose the best regions for willow biomass production in Canada as well as the best sites within those regions. Also discussed in this chapter are the nutritional demands of willow. By highlighting these growth requirements this chapter allows for forecasts of the potential of amendments and management techniques to mitigate nutritional deficiencies at some sites thereby maximizing productivity and use of resources.

3. CONTROLS OF EDAPHIC CONDITIONS ON SITE QUALITY FOR WILLOW (*SALIX* SPP.) PLANTATIONS ACROSS A LARGE CLIMATIC GRADIENT IN CANADA

3.1 Abstract

Willow has been identified as a possible species for supplying large quantities of biomass for bioenergy and wood fibers in Canada. However, there is a need to know how willow responds to the soils and climate of the prairie and southern Ontario regions before site selection occurs. Soil, foliar, and climatic variables were measured along with productivity at nine "first rotation" *S. purpurea* 'Hotel' plantations. Correlation analysis was used to identify those parameters having the most influence on willow growth. Equations describing the relationships between soil and climate and productivity were produced from measurable site variables via regression. The acid-base status of the soil as dictated by bulk elemental composition, in particular the presence of Ca, was found to be the dominant control ($r^2 = 0.87$, $p < 0.001$ total Ca vs. productivity) despite large regional differences in climate. From a nutritional standpoint, sites with more available N ($r^2 = 0.45$, $p < 0.001$ foliar N vs. productivity with adequate moisture) and K ($r^2 = 0.51$, $p < 0.001$ foliar K vs. productivity) also had greater growth. Productivity was found to be limited by water availability at only the two lowest productivity prairie sites. While soil total Ca concentration, pH and total organic C concentration were all generally correlated with higher productivity, each site had its own unique combination of limiting factors such that as one was removed another would become limiting: severe water stress > N and K deficiencies > Mg and P deficiencies.

3.2 Introduction

3.2.1 Willow grown as SRIC

There is expanding interest in the use of fast growing tree species for both wood fiber and fuel. Using fast growing species in dedicated plantations reduces pressures on natural forests and can substantially reduce transportation costs. The Canadian prairies could provide an extensive land base for growing trees in a plantation setting. However, there are challenges for tree growth as this region is prone to short dry growing seasons and harsh cold winters (Padbury et al., 2002).

As such, native tree growth is often limited to depressions, north facing slopes and more northern regions where evapotranspiration rates are decreased by cooler temperatures (Padbury et al., 2002; Richards and Fung, 1969). Poplar (*Populus* spp.) and willow (*Salix* spp.) are two native genera that have been identified as having potential to cope with these unfavorable conditions (Padbury et al., 2002; Richards and Fung, 1969) and return economically viable yields despite being water demanding. Poplar has been widely used in the Canadian prairies in shelterbelts to prevent soil erosion and conserve soil moisture. Tree yields are greater in British Columbia, Ontario and Quebec than in the prairie provinces, but the prairies offer a larger and less expensive land base (McKenney et al., 2004). Willow is relatively new to agroforestry use in Canada but it does offer some advantages. For one, there is tremendous natural diversity of willow, with over 450 species having been identified (Argus, 1997), which provides opportunity for breeding programs to tailor hybrids for specific needs (Kopp et al., 2001a). Willow regrows vegetatively and responds well to coppicing (Volk et al., 2004), which means that rotation lengths can be short (typically 3–5 years), depending on planting and management style, and the trees will stay vigorous for several rotations (Mitchell et al., 1999; Volk et al., 2004). This addresses the need for producers to recover costs as soon as possible. It also enables a producer to harvest stems damaged by pests and extreme weather and restore vigor to the plant in the process. Thus, willow is well suited to short rotation intensive culture (SRIC) on the Canadian prairies.

If willow is to be planted as SRIC in Canada, then knowledge of how it will respond, in terms of productivity, needs to be examined to ensure that plantations are established in appropriate locations and productivity can be maintained well into the future without overuse of irrigation and nutrient amendments that would unnecessarily increase the water and energy demands of the system (Bhardwaj et al., 2011). Site quality index, which is the height of the dominant trees for a given location and management regime at an arbitrary age is the most commonly used estimate of productivity in forestry (Ung et al., 2001). This system can be adapted to suit willow more effectively by substituting measures of biomass for height. Even so, because plantations are to be established in the context of afforestation, there are no pre-existing willow trees to measure site quality index for most sites before planting. Therefore, it is

necessary to examine the underlying soil and climatic factors controlling site productivity for willow grown under a wide range of Canadian soil and climatic conditions. Specific limiting factors must be identified, after which the relationships between these variables and willow growth can be used to assess the quality of a site without directly measuring the traditional site quality index.

3.2.2 Willow site quality

Research thus far has concluded that willow grows best in loamy soils with a well developed structure and a rooting depth of 45 cm or more (Abrahamson et al., 2002). Ledin and Willebrand (1995) concluded that, in general, SRIC requires a soil with a minimum depth of 1 m. Willow has been found to establish slowly on soils with high clay content but it has been suggested that productivity may be greater in these soils in successive rotations (Abrahamson et al., 2002). Clayey soils are difficult to penetrate for young roots but have a much larger exchange capacity, thereby maintaining better nutrition of rapidly growing trees. Excessively well or poorly drained soils or soils with a pH lower than 5.5 or higher than 8.0 have been shown to limit productivity (Abrahamson et al., 2002; Mitchell et al., 1999).

Willow growth in Canada is on average lower than that achieved in the United States and parts of Europe owing in large part to a limited growing season (Mitchell et al., 1999). Moreover, the fast growing nature of willow requires a steady supply of water and nutrients for optimal growth. Although Lindroth and Cienciala (1996) reported a relatively high water use efficiency for willow (6.3 g kg^{-1}), a moderate value for annual precipitation on the Canadian prairies of 430 mm would produce a maximum of only 10 Mg ha^{-1} yr^{-1}. Thus, the low amount of precipitation in the Canadian prairies has been expected to greatly hinder willow growth such that the importance of fertility is diminished. Additionally, Guidi et al. (2008) concluded that increasing the available nutrients for willow also increases transpiration. In a water limited region, this would translate to underutilization.

Willow has frequently been shown to respond favorably to fertilization, particularly with N (Adegbidi et al., 2003) both from synthetic and organic amendments (Labrecque et al., 1998). However, when planted on fertile arable land, it may be up to 10 years before any response to

fertilizer occurs (Mitchell et al., 1999). As such, amendments should be applied only where there is a need and should not be a common prescription for every site. What needs further examination, especially in Canada, is the potential for willow growth given soil and climatic factors at a variety of sites and an estimate of the ability of these sites to achieve optimal nutrient status before adding amendments.

There are many factors including soil pH and ionic concentrations of other nutrients that affect the ability of a plant to take up essential nutrients. Adegbidi et al. (2003) found that although willow initially responded similarly to a single input of either N-fertilizer or organic amendments (sewage sludge or manure), the increase in growth associated with the organic amendments continued over a longer period of time. Maintaining a continual supply of the required nutrients for willow growth depends upon the nutrients present within the soil and its capacity to store and exchange ions and water. Thus, soil C and organic matter is critical for maintaining beneficial soil properties. It is therefore important to examine both the response in growth to soil and climate variables as well as how different soils are meeting the nutritional needs of willow by examining the nutrient concentrations, specifically in leaves of willow directly.

Simon et al. (1990) described foliar nutrient levels of willow leaves, stems and whole plants grown with no nutrient limitations by employing a nutrient solution maintained at optimum levels for the species. Although there was considerable variation, particularly with N, this does provide a useful reference for willow nutrition at its optimum. Foliar N, P, and potassium (K) concentrations were respectively 28.4, 1.8, and 19.0 mg g^{-1} for *S. eriocephala*, 35.4, 2.3 and 22.0 mg g^{-1} for *S. exigua*, and 28.0, 1.9, and 23.4 mg g^{-1} of dry weight for *S. lucida*. Similarly, Kopinga and van den Burg (1995) reported optimal foliar nutrient concentrations for willow at N > 22, P > 2.1, K > 19, and Mg > 3.0 mg g^{-1}. These values are much lower than those reported by Cornelissen et al. (1997) who found *S. caprea* to have the highest N (43.0 mg g^{-1}), P (6.53 mg g^{-1}), and K (32.4 mg g^{-1}) foliar concentrations of 34 temperate deciduous and coniferous trees species. The N and K requirements of *Salix* species identified by Simon et al. (1990) and Kopinga and van den Burg (1995) would similarly be at the high end of nutrient levels in Cornelissen et al. (1997) (N ranges from 13.1-32.9 mg g^{-1} and K from 6.1-21.7 mg g^{-1}

excluding *S. caprea*), though P concentrations were more moderate (1.08-5.71 mg g^{-1} excluding *S. caprea*). The final nutrient examined in this study, (Ca), can exhibit considerable variation in concentrations in leaves with species and with growth stage (Jones, 1998). The higher demand for nutrients of willow (Mitchell et al., 1999; Simon et al., 1990) compared to many other temperate tree species (Alriksson and Eriksson, 1998; Bélanger et al., 2004; Cornelissen et al., 1997) may mean that productivity is controlled more by soils than by climate unlike other temperate species (Hamel et al., 2004; Hogg et al., 2005; Post and Curtis, 1970; Ung et al., 2001). It is therefore hypothesized that site quality will be dependent primarily on soil factors and that climate will be responsible for less of the variability in production observed between sites. This pattern could be influenced by geographic scale. For example, regional-scale studies (Hogg et al., 2005; Ung et al., 2001), having large climatic gradients, place more importance on the impact of climate on tree growth, whereas local-scale studies (Pinno and Bélanger, 2011; Pinno et al., 2009), having much smaller climate gradients, place more emphasis on the effects of soils—in particular those factors that dictate nutrient availability—on tree productivity.

Despite increasing interest in willow as a SRIC crop in the Canadian prairies there is little available data for determining suitable site quality. The objective was therefore to measure soil and climate variables as well as productivity and nutrition across a broad range of sites in Canada to determine which set of conditions are conducive to greater growth. It is hypothesized that, due to willow's high nutrient demand and the broad range of soil present in Canada, soil, and in particular nutrient status, will be dominant over climate in controlling willow productivity. In testing this hypothesis, high and low site quality for willow production are characterized.

3.3 Materials and Methods

There is a broad range of climate conditions across the Canadian prairies and southern Ontario and as such this is expected to have an impact on willow growth. Included in this study are nine sites following a transect from the northwestern to southeastern boundaries of the Canadian prairies and extending into southern Ontario (Table 3.1). Much of the climate diversity of the prairie region is included in this study, with the exception of the driest regions of southern Alberta and Saskatchewan. As a generalization, there are longer and warmer growing seasons

and more precipitation towards the southeast. At the northern bounds of the Canadian prairies, evapotranspiration decreases and in turn so does the water deficit, leading to the growth of native forests despite only modest gains in precipitation compared to the southern prairies.

Table 3.1: Site names, locations, history, soil texture, soil pH, bulk density, precipitation and harvested biomass summary data for nine *S. purpurea* 'Hotel' plantations included in this study.

Site (ID)	Geographic Coordinates	Site History	Soil Texture	Soil pH (SE)	Bulk Density (SE)	Mean Annual Precipitation (SE)†		Year Planted/ Harvested	Harvested Biomass (SE)
						30 Year Mean (SE)	Year Planted to Year Harvested (SE)		
						——— mm ———			Mg ha^{-1}
Ellerslie Research Station, AB (ELL)	53°25'N 113°31'W	Cereal crops	Silty Clay Loam	6.0 (0.1)	1.09 (0.01)	444 (14)	404 (43)	2005/ 2007	N/A¶
Smoky Lake, AB (SMO)	54°07'N 112°24'W	Tree nursery‡	Sandy Loam	5.1 (0.1)	1.51 (0.02)	432 (13)	412 (27)	2005/ 2007	5.4 (0.1)
Lakeshore Tree Nursery, SK (LAK)	52°00'N 106°45'W	Pasture	Silty Clay Loam	8.3 (0.0)	1.24 (0.02)	349 (16)	454 (53)	2005/ 2007	18.4 (1.2)
University of Saskatchewan, SK (UOS)	52°07'N 106°36'W	Cereal crops	Clay	6.6 (0.1)	1.24 (0.02)	364 (17)	382 (74)	2006/ 2008	5.3 (0.8)
Portage la Prairie, MB (POR)	49°57'N 98°10'W	Vegetables	Silt Loam	8.3 (0.0)	1.09 (0.01)	537 (17)	523 (88)	2005/ 2007	22.8 (3.2)
Bird's Hill, MB (BIR)	50°00'N 97°00'W	Pasture	Heavy Clay	8.3 (0.1)	1.18 (0.01)	560 (21)	538 (91)	2005/ 2007	N/A¶
Sault Ste Marie, ON (SSM)	46°32'N 84°24'W	Managed forest	Sandy Clay Loam	5.4 (0.1)	1.39 (0.02)	914 (26)	826 (56)	2006/ 2008	6.1 (0.3)
Guelph, ON (GUE)	43°33'N 80°13'W	Turf grass	Clay Loam	6.9 (0.0)	1.29 (0.03)	894 (26)	971 (143)	2005§/ 2008	9.5 (2.5)
Pickering, ON (PIC)	44°00'N 79°01'W	Idle / Failed plantation	Clay	6.8 (0.2)	1.22 (0.03)	887 (18)	948 (131)	2006/ 2008	14.0 (2.1)

† Modeled using BioSIM.
‡ Unable to find ideal optimal match to previous land use; used surrounding forest.
§ Coppiced after first year; three years of aboveground biomass.
¶ Harvested previously such that comparable data is unavailable.

Climate data for the years in which the trees grew were modeled using the BioSIM model (Régnière, 1996), which typically yields a coefficient >0.98 between measured and modeled values (Régnière and Bolstad, 1994). The BioSIM model uses data collected from surrounding weather stations to model site specific conditions where weather stations do not exist. Degree days were calculated at both the 5°C and 10°C base. In addition, total annual precipitation, summer precipitation vapor pressure deficit, potential evapotranspiration and aridity index were all modeled in order to better understand how willow growth was affected by climate.

At each site, the ’Hotel‘ clone of *Salix purpurea* was planted in triple rows with 60 cm spacing between trees and 200 cm between rows. This clone was selected largely for logistical reasons, as planting stock (unrooted cuttings) was readily available. Expected *S. purpurea* ‘Hotel’ yields are moderate to low when compared to other clones bred as bioenergy feedstock, however, this clone has been relatively hardy in the Canadian climate and resistant to herbivore and disease damage. Soil and foliar samples were collected during the third year of the first rotation from five plots at each plantation. Plots were 3.2 m by 1.8 m and contained nine trees according to the original planting as well as a proportional representation of in row and between row area.

Soil and leaf samples were collected at all sites. However, of the nine sites included in this study for nutritional analysis, two (ELL, BIR) were coppiced a year before sampling which left only seven sites that had accumulated three years of growth (which is the general rotation length). GUE had been coppiced after the first year of growth but was allowed to still accumulate biomass for three years afterwards. While coppicing is typically implemented with the purpose of increasing biomass production via promoting branching to form multiple stems, *S. purpurea* ‘Hotel‘ branches readily and as such there were no strong morphological differences between GUE and other sites. GUE willow did have an advantage in that the root system had an extra year of growth compared to other sites but it was deemed that this difference was within the variability associated with variance in management across the large scale of this study. Thus, for the sake of studying the factors regulating aboveground biomass production, only sites with three years aboveground biomass accumulation were considered (n=35), whereas all sites (n=45) were included for linking soil fertility to foliar nutrition.

Within each plot, soils were sampled at 0-20 cm and 20-40 cm increments (3 replicates). Soils were analyzed for total organic C, total N, inorganic N forms (NO_3 and NH_4), bulk elemental composition (e.g. total Ca, total K and total Al), acid leachable P, exchangeable K, Ca, Mg, sodium (Na), iron (Fe) and aluminum (Al), pH, sand, silt, and clay. Soil C and N were determined by combustion at 800°C and infrared detection on a LECO CNS-2000 (LECO Corporation, St. Joseph, MI). Nitrate and NH_4 were extracted using 2.0 *M* KCl and analyzed on a Technicon Auto-Analyzer (Technicon Instruments Corporation, Tarrytown, NY). Phosphorus (total P) was leached with H_2SO_4 and measured with a SmartChem Instrument (Mandel Scientific Company Inc., Guelph, ON). Exchangeable K, Ca, Mg, Na, Fe, and Al were extracted using a 0.1 *M* $BaCl_2$ solution (Hendershot et al., 2008) and analyzed by atomic emission (K, Na) and absorption (Ca, Mg, Fe, Al) (SpectraAA 220, Varian Analytical Instruments). Soil pH was measured in a 2:1 water to soil suspension. Particle size distribution (soil texture) was analyzed on a Horiba Partica LA-950 laser particle analyzer and corrected for clay content using the pipette method (Kroetsch and Wang, 2008). The bulk elemental composition of soils from 0-20 cm was determined by X-ray fluorescence spectroscopy following the procedure in Bélanger et al. (2004).

Fifty upper canopy leaves were randomly collected from within each plot in early August of the year harvested (i.e. 2007 and 2008), weighed, dried and ground in bulk for foliar analysis. Stems were harvested and weighed in the field for fresh weight once leaves had senesced. In the event that total leaf fall had not yet occurred, leaves were first removed manually before harvesting stems. Fresh weight of the stems was measured in the field. A 1-2 kg subsample, incorporating whole stems, was dried and reweighed to convert fresh weight to dry weight. The stem subsamples were chipped and mixed to ensure homogeneity. Two samples of approximately 40 g were then ground in stages to produce a fine homogeneous powder. Leaves and stems were analyzed for concentrations of C, N, P, K, Ca, Mg, and Na. Carbon and N of leaves were determined by infrared detection on a LECO CNS-2000 at 800°C. Phosphorus, K, Ca, Mg, and Na concentrations of leaves and stems were determined from a concentrated H_2SO_4-H_2O_2 digest (Kalra and Maynard, 1999). Concentrations of PO_4 were measured with a SmartChem Instrument (Mandel Scientific Company Inc., Guelph, ON). Potassium, Ca, Mg, Na were

determined by atomic absorption (Ca, Mg, Fe, Al) and emission (K, Na) spectroscopy (SpectraAA 220, Varian Analytical Instruments).

The $\delta^{13}C$ signature (the ratio of ^{13}C to ^{12}C as compared to a standard) was used as an indicator of water stress separately from growth response to precipitation (Warren et al., 2001). Stems were very finely ground as a mix such that percentage from any one year was proportional to the growth increment that year (i.e. first year of growth is under represented) and analyzed using a RoboPrep Sample Converter interfaced with a TracerMass Stable Isotope Detector (Europa Scientific, Crewe, UK). During the process of mass spectrometry, total C and total N contents of the sample were simultaneously determined.

Relationships between harvested biomass and each soil, plant nutrient or climate variable were determined using the Pearson product-moment correlation. However, many of the relationships were non-linear in which case non-linear regression was used. Stepwise and forward multiple linear regression was used to determine which measured parameters would work best together to predict productivity at the 35 study plots. The maximum number of variables selected in the multiple regression analysis was set at three in the interest of achieving parsimonious relationships while still utilizing the predictive capability of the analyses (Pinno and Bélanger, 2011). Relationships at $P = 0.05$ were considered significant. Statistical analyses were carried out with CoStat (CoHort Software, Monterey, CA).

3.4 Results

Across sites soils were highly varied from sandy loam (pH = 5.1) to heavy clay (pH = 8.3). Nutrient content of soil was similarly wide-ranging (total N = 1.75-3.03 mg g^{-1}, acid leachable P = 0.35-1.15 mg g^{-1}, exchangeable K 0.09-1.33 $cmol_c$ kg^{-1}). The large geographic scale of this study also meant climate and weather was also very diverse (mean annual precipitation = 382-971 mm, degree days (base 5°C = 1330-2300 °C day). Therefore it is unsurprising that there was a large range in productivity (5.3-22.8 Mg ha^{-1}) present across sites in response to the diversity of site characteristics (Table 3.1). In general, willow with the greatest productivity tended to be on medium to fine textured calcareous soils. Sites with sandy soils, very low precipitation or a combination of both produced the lowest yields.

Few climate variables were correlated with productivity (Table 3.2). There was no relationship between willow productivity and annual or summer precipitation over the course of three years. There was however a positive correlation (r = 0.752, *P* = 0.051) between summer precipitation in the first two years of growth.

Table 3.2: Pearson product-moment correlations for climate variables modeled with BioSIM and harvested biomass for seven *S. purpurea* 'Hotel' plantations.

	r	*P*
Degree days (base 5°C)	0.398	0.377
Degree days (base 10°C)	0.425	0.341
Annual precipitation	-0.058	0.902
Summer precipitation (3 years)	0.256	0.579
Summer precipitation (First 2 years)	0.752	0.051
Thornwaite potential evapotranspiration	0.543	0.208
Standard Thornwaite potential evapotranspiration	0.343	0.452
Vapor pressure deficit	-0.080	0.865
Aridity index	0.236	0.446

Different plant species have unique $\delta^{13}C$ signatures based on their intrinsic water use efficiency and photosynthetic pathway (i.e., C_3 vs. C_4 plants). This means that there is no absolute baseline $\delta^{13}C$ signature indicating water stress; rather that as the $\delta^{13}C$ increases (becomes less negative) water use efficiency increases in response to water scarcity and plants are likely more water stressed than others of the same species. Examination of $\delta^{13}C$ values of stems confirms that there is no simple relationship between productivity and water stress across all sites (Figure 3.1). Rather, there are two distinct groups: SMO and UOS were the only two sites where moisture limitations crossed a threshold severe enough to limit productivity and cause these sites to have the highest $\delta^{13}C$ values. At the other sites, factors other than water stress were more limiting and in fact $\delta^{13}C$ values across these sites were positively correlated with harvestable biomass.

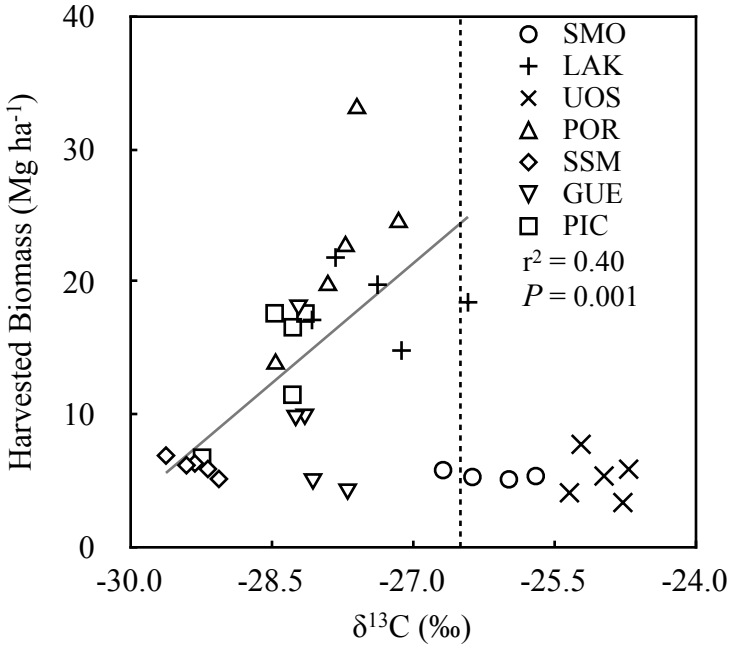

Figure 3.1: $\delta^{13}C$ of stems as an estimate of water stress versus harvested biomass for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC). Less negative $\delta^{13}C$ values (towards right) indicate increasing water stress. Dashed line represents approximate threshold for acute water stress. Regression excludes SMO and UOS as these sites are beyond the threshold for acute water stress and therefore are a distinct population from the remaining dataset.

Site quality was strongly correlated with bulk soil chemical composition (Table 3.3). Total Ca (Figure 3.2a) was the best single indicator of willow productivity as were pH (Figure 2c) and (ΣTotal Ca+Mg)/(ΣTotal Si+Al+Fe), an indicator of the acid-base status of the soil (Birkeland, 1999) (Figure 3.2d). Soil organic C was another good general indicator of productivity (Figure 3.3). Additionally, soil texture was correlated with productivity as willow grew best on medium to fine textured soils with adequate drainage (Table 3.3, Figure 3.4).

Table 3.3: Pearson product-moment correlations for measured soil variables and harvested biomass for seven *S. purpurea* 'Hotel' plantations.

	0 - 20 cm		20 - 40 cm	
	r	*P*	r	*P*
pH	0.777	0.000	0.690	0.000
Organic C	0.660	0.000	0.743	0.000
Total N	0.435	0.009	0.172	0.324
C:N ratio	0.370	0.029	0.510	0.002
H_2SO_4 extractable P	0.421	0.012	0.371	0.029
Bulk chemistry				
Total Ca	0.891	0.000	--†	--
Total Mg	0.825	0.000	--	--
Total Al	0.293	0.088	--	--
Total Fe	0.288	0.093	--	--
Total Si	-0.670	0.000	--	--
Total K	0.265	0.123	--	--
Total P	0.518	0.001	--	--
Exchangeable Cations				
K	0.372	0.028	0.072	0.679
Ca	0.714	0.000	0.604	0.000
Mg	-0.383	0.023	-0.378	0.025
Na	-0.338	0.047	-0.350	0.039
Fe	-0.388	0.021	-0.404	0.016
Al	-0.446	0.007	-0.355	0.036
NO_3^-	-0.169	0.331	-0.095	0.588
NH_4^+	-0.412	0.014	-0.317	0.063
Sand content	-0.547	0.001	-0.104	0.554
Silt content	0.713	0.000	0.485	0.003
Clay content	0.083	0.635	-0.283	0.099

† not included in analyses.

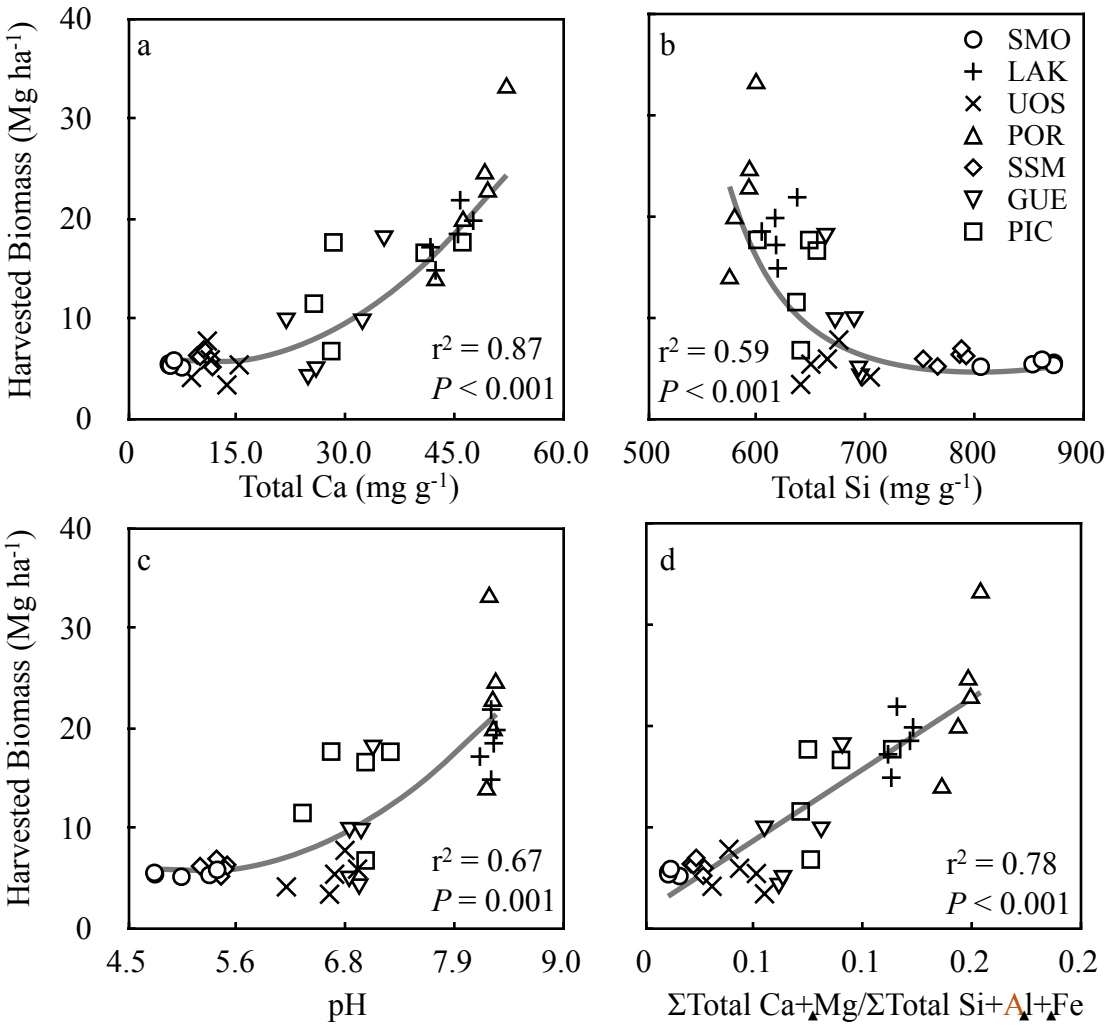

Figure 3.2: Effect of Total Ca (a), Total Si (b), pH (c) and Total Ca+ Mg/Total Si + A l+ Fe (d) of top 20 cm of soil on harvested biomass for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC)

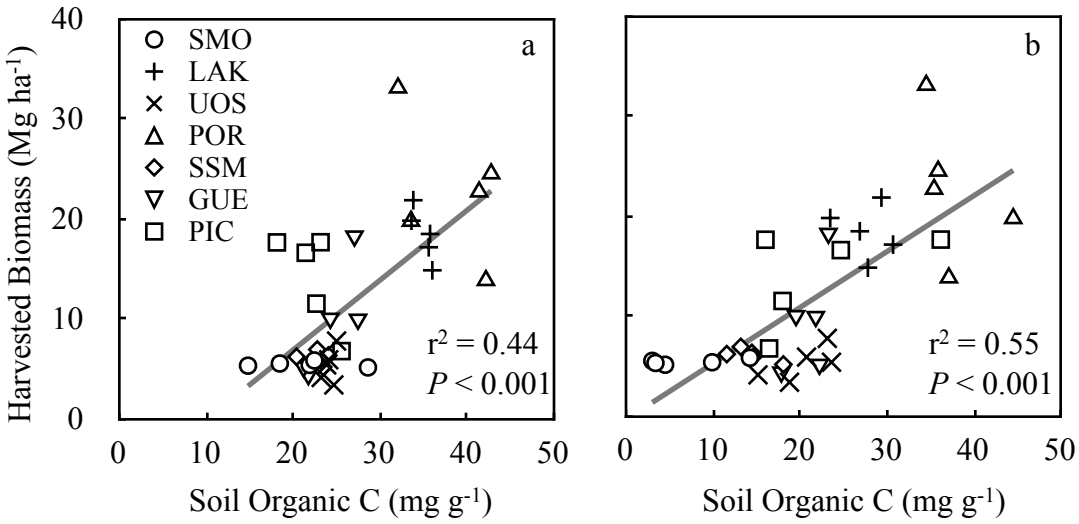

Figure 3.3: Relationship between organic soil C at 0-20 cm (a) and 20-40 cm (b) and harvested biomass for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC).

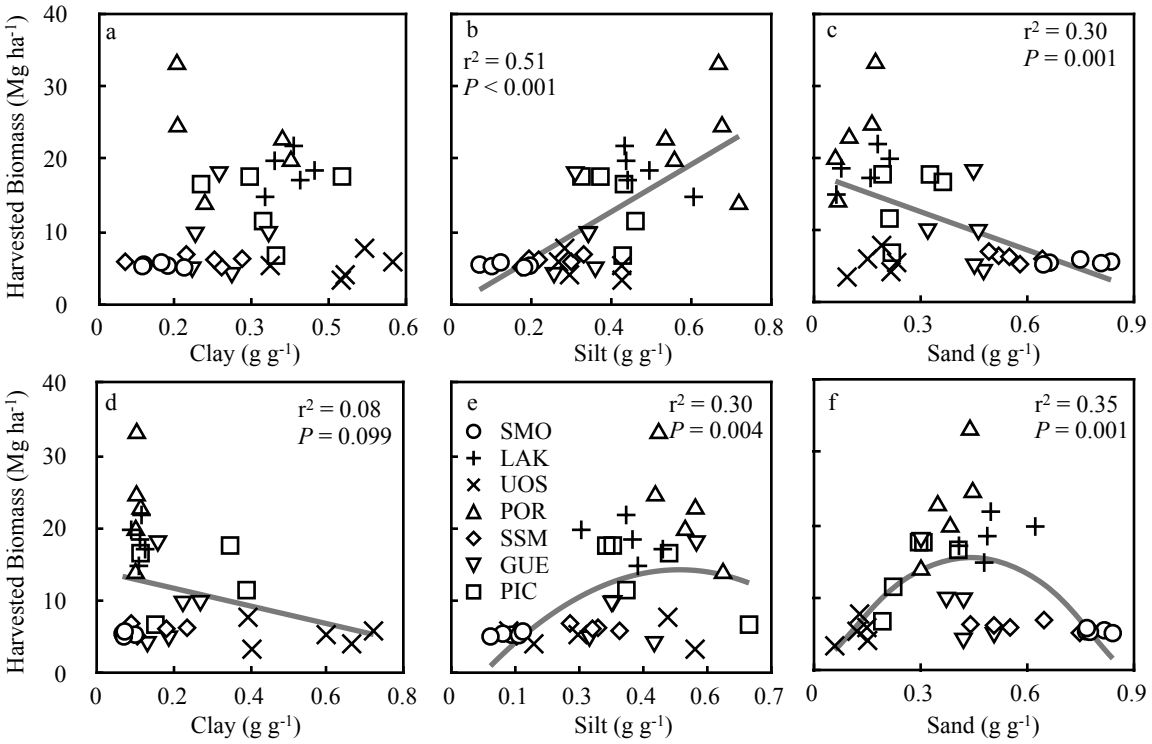

Figure 3.4: Relationship between soil texture in 0-20 cm (a, b, c) and 20-40 cm (d, e, f) and harvested biomass for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC).

Foliar concentrations of N (Figure 3.5), P (Figure 3.6), and K (Figure 3.7) were related to the measured soil variables while Mg and Ca were not. When considering the entire dataset, positive correlations between foliar concentrations and productivity were found for K (Figure 3.7) and Ca (Figure 3.8) (Table 3.4) and negative for Mg. With water stressed sites removed positive correlations existed with N and K but not for Ca. Magnesium was only positively correlated with productivity at LAK, POR and PIC sites (Figure 3.9).

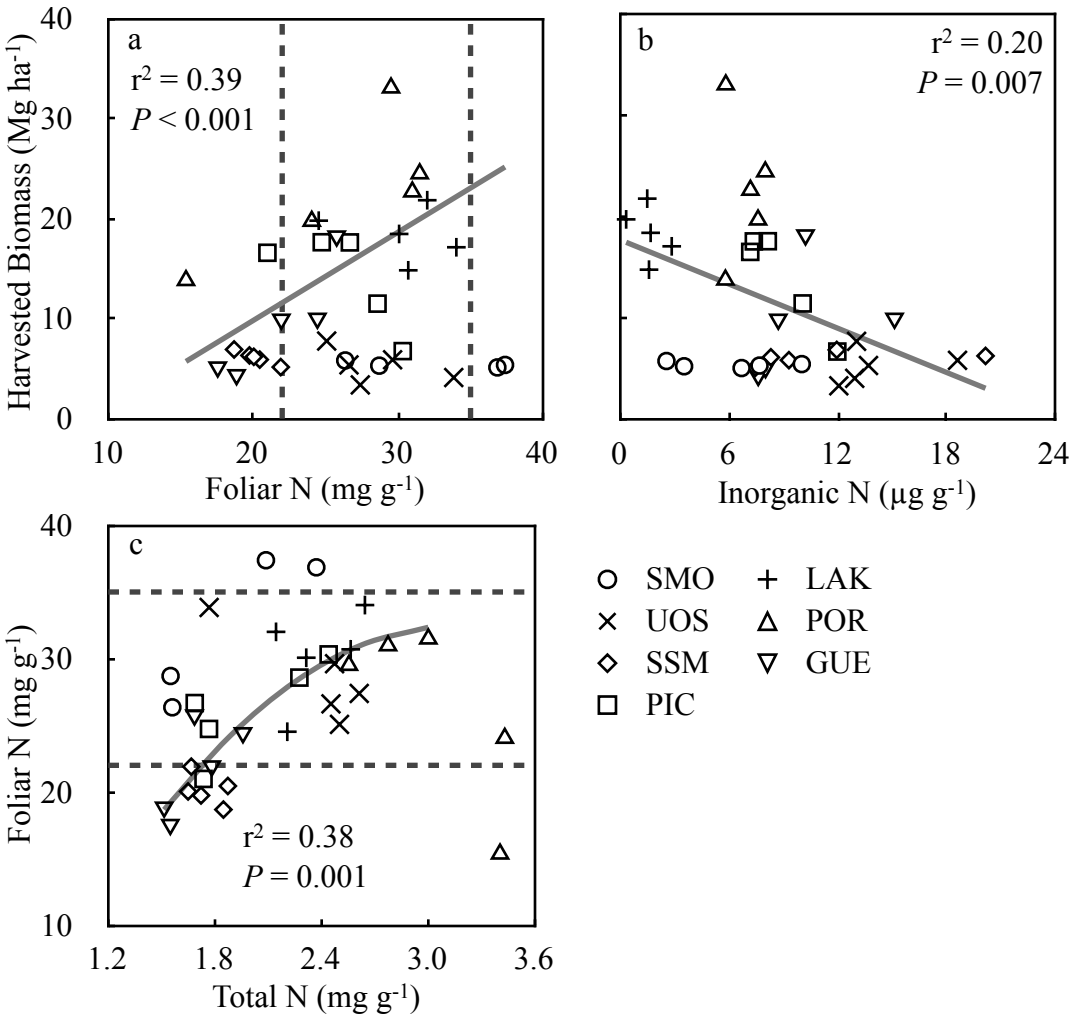

Figure 3.5: Relationship between N availability and productivity expressed as foliar N (a) and soil inorganic N (0-20 cm) (b) vs. harvested biomass and total N vs. foliar N (0-20 cm) (c) for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC). Due to severe water stress, UOS and SMO sites do not share benefit from increasing N uptake and as such are excluded from the regression dataset. Total N vs. Foliar N regression (c) excludes points past 3.1 mg g^{-1} Total N. Dashed lines represent low and high of range of optimal concentrations of foliar N for other *Salix* species (22-35 mg g^{-1}) as reported by Simon et al. (1990) and Kopinga and van den Burg (1995).

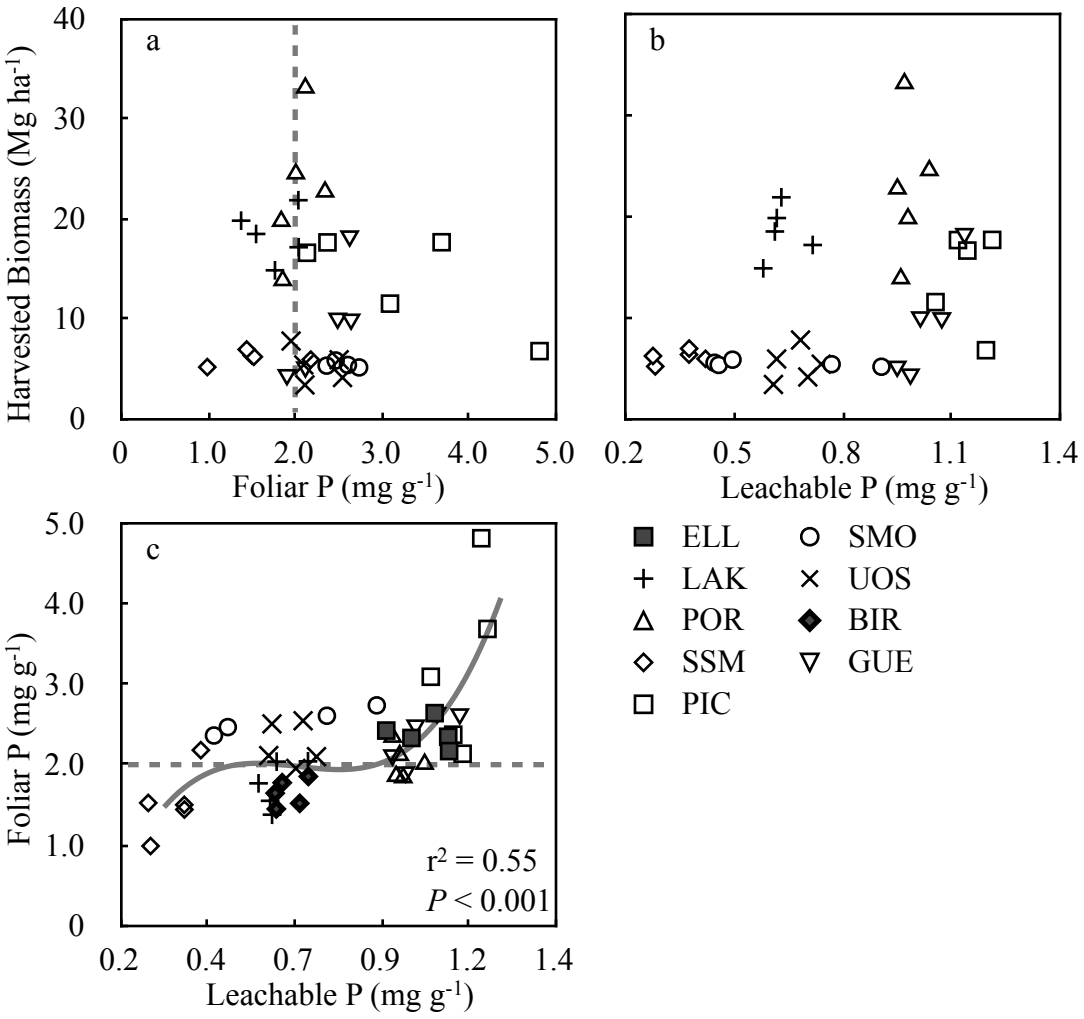

Figure 3.6: Effects of foliar P (a) and H_2SO_4 leachable P concentration (0-20 cm)(b) on harvested biomass for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC) and uptake as soil P content (0-20 cm) vs. foliar P(c) for nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Dashed line represents apparent approximate sufficiency for P (Simon et al., 1990).

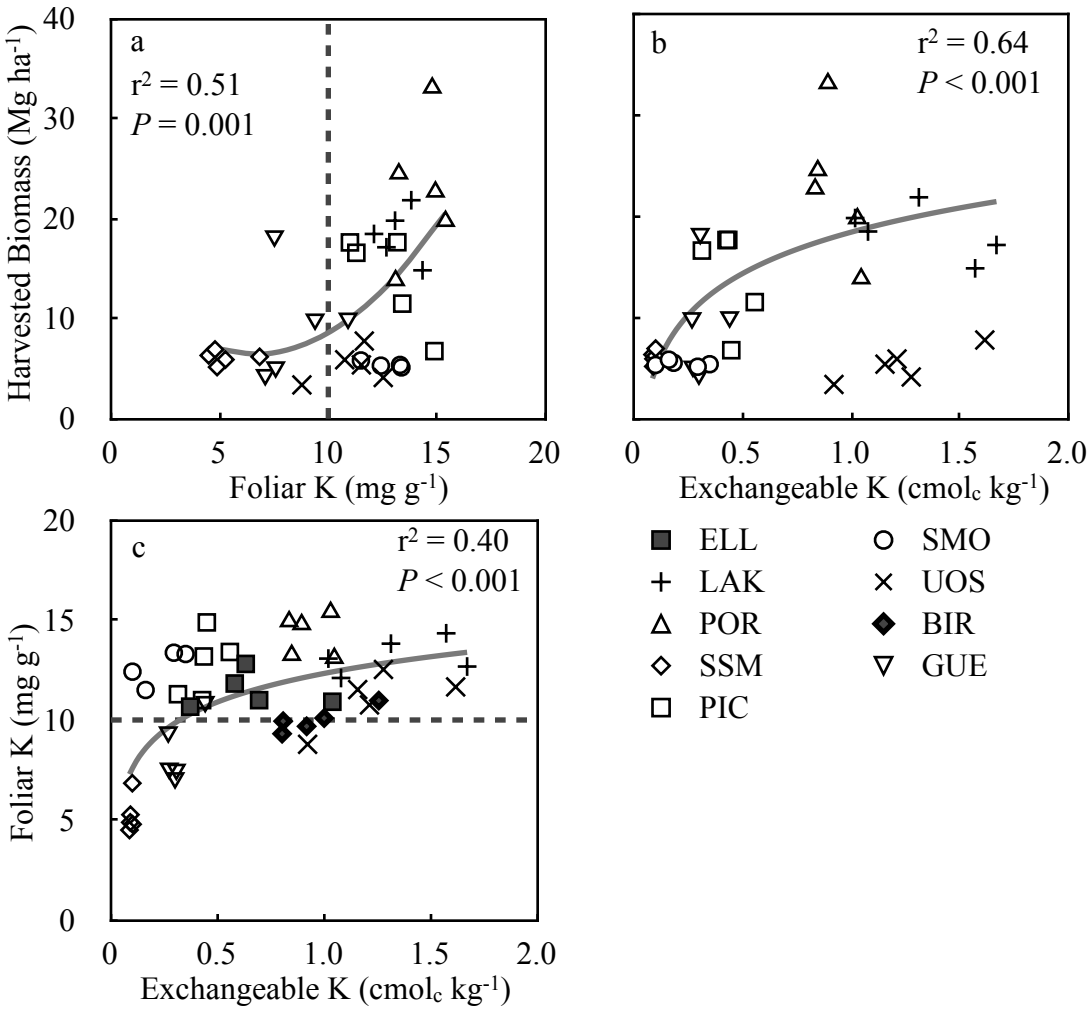

Figure 3.7: Relationship between K availability and productivity expressed as from increased foliar K (a) and exchangeable K (0-20 cm) (b) vs. harvested biomass for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC) and exchangeable K (0-20 cm) vs. foliar K (c) for nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Due to severe water stress, UOS and SMO sites do not share benefit from increasing K uptake and as such are excluded from the regression datasets (a, b). Minimum threshold for foliar K (10 mg g-1) indicated by dashed lines.

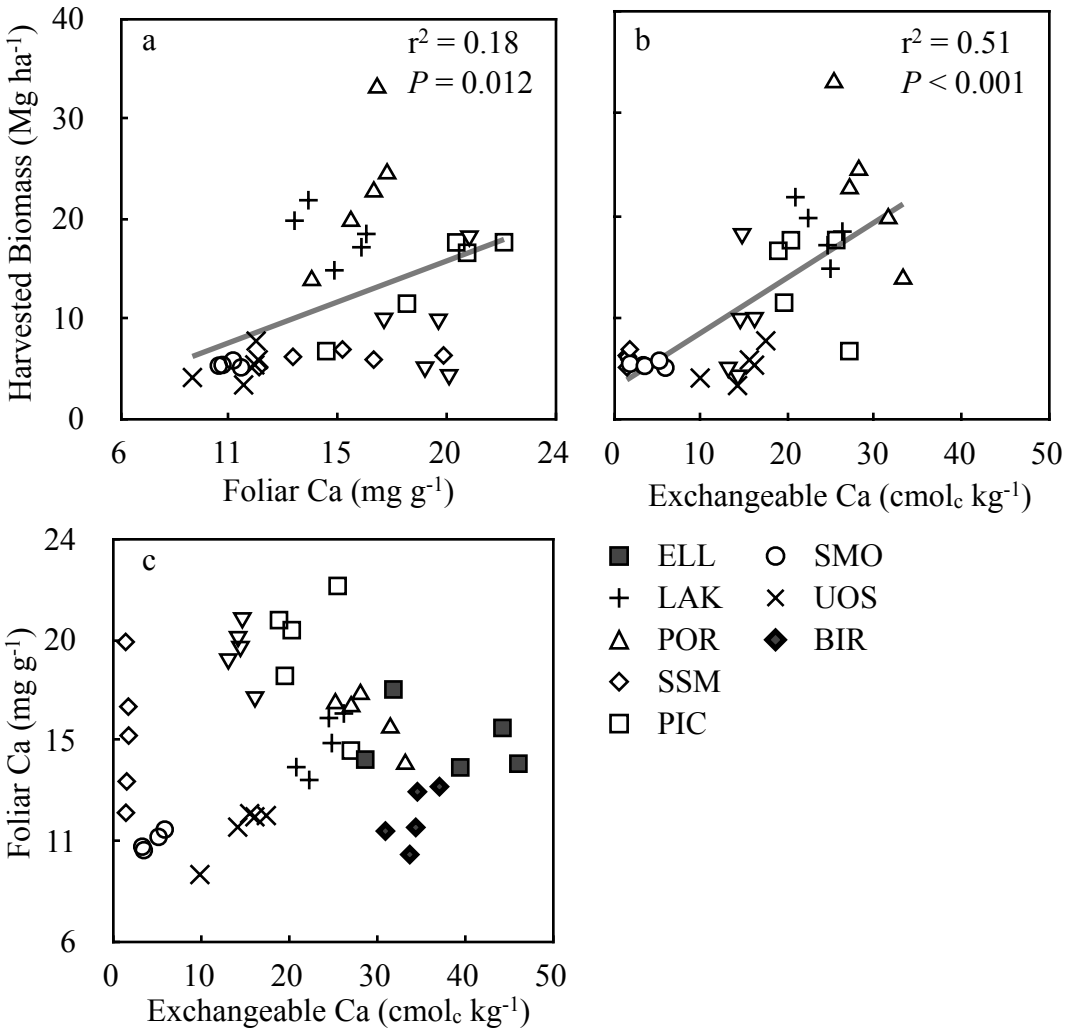

Figure 3.8: Effect of foliar Ca (a) and soil Ca (0-20 cm) (b) on harvested biomass for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC) and uptake as soil Ca concentration (0-20 cm) vs. foliar Ca (c) for nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC).

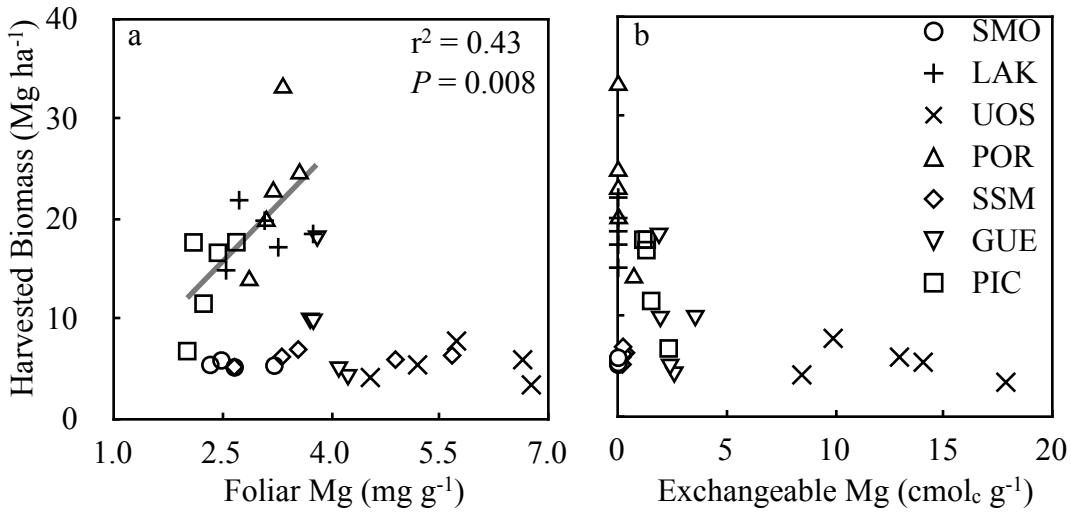

Figure 3.9: Growth response (harvested biomass) to differing concentrations of foliar Mg (a) and exchangeable soil Mg concentration (0-20 cm) (b) for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC). Due to severe water stress at UOS and SMO sites and K and N deficiencies at SSM and GUE sites, they do not share benefit from increasing Mg uptake and as such are excluded from the regression dataset.

Table 3.4: Pearson product-moment correlations for measured foliar variables and productivity for seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC).

	All Sites (SMO, LAK, UOS, POR, SSM, GUE, PIC)		Water Stressed Sites Excluded (LAK, POR, SSM, GUE, PIC)		Water, N, K Stressed Sites Excluded (LAK, POR, PIC)	
	r	*P*	r	*P*	r	*P*
Stems						
$\delta^{13}C$	-0.214	0.217				
Leaves						
Nitrogen	0.193	0.274	0.624	0.001		
Potassium	0.515	0.002	0.709	0.000		
Calcium	0.427	0.012	0.044	0.833	0.038	0.893
Magnesium	-0.355	0.039	-0.291	0.158	0.654	0.008
Phosphorus	-0.076	0.670	-0.004	0.986	-0.520	0.047

None of the multiple linear regression models calculated via stepwise and forward regression were able to exceed the strength of the relationship described by a second order polynomial with total Ca (Figure 3.2). Several models could be created by combining different

variables with total Ca but these yielded slightly lower coefficients of determination (see Appendix). In terms of secondary predictors, there is both a high degree of covariance among variables and relatively little room for improvement of the models such that multiple linear regressions, both stepwise and forward, were not able to adequately identify the most important nutritional or climatic requirements of willow.

3.5 Discussion

Degree days are useful in estimating the potential for plant productivity from a temperature standpoint (Wang, 1960) and have been shown to be positively correlated with willow growth ($r^2 > 0.95$) (Kopp et al., 2001b). Because of the large climatic gradient created in this study, it was expected that greater annual precipitation and a longer growing season as reflected by degree days would have been somewhat important for determining willow productivity. However, in this study, degree days did not explain the variability in average site productivity. This is at odds with most studies examining tree growth at the regional scale (Post and Curtis, 1970; Lindroth and Båth, 1999; Ung et al., 2001; Hamel et al., 2004, Hogg et al., 2005) and may be related to the high water and nutrient requirements of *S. purpurea* 'Hotel' or a possible effect of photoperiod across the range in latitude that was beyond the scope of this study.

Likewise, other climate variable had poor correlations with growth. Vapor pressure deficit, which is the difference between the actual water content of the air and the potential content of the air at a relative humidity of 100%, is a good measure of the atmospheric control on transpiration. Potential evapotranspiration is similarly reflective of potential plant activity as it increases with increasing available solar radiation but can cause water stress if precipitation cannot meet demands. Aridity index, which is based on the ratio of precipitation to potential evapotranspiration, takes into consideration both the supply and use of water. However, none of these variables satisfactorily explained the variability in biomass production.

In fact, soil properties were the dominant predictors of willow growth in this study. This suggests that, even at the regional scale, the growth of *S. purpurea* 'Hotel' is governed in large part by the capacity of soils to retain water and nutrients and that climate plays a lesser role.

Thus, site selection based on soil quality can result in minimizing the need for inputs such as irrigation and fertilizers (Bhardwaj et al., 2011), thereby keeping the water and energy footprints to a minimum and maximizing the environmental benefits of willow biomass production.

3.5.1 Water availability

The ^{13}C:^{12}C signature of willow stems was correlated with total precipitation over three years (r = -0.789, P = 0.028) but not summer precipitation over the first two years growth (r = -0.472, P = 0.276). This suggests that the most critical first two years may be underrepresented relative to their importance in determining productivity. Figure 3.1 does indicate that as productivity rose from other factors (i.e., nutrition), photosynthetic rate increased and likely water use efficiency as well—as faster growing plants exerted more pressure on dwindling moisture supplies (Moukoumi et al., 2012). Both of these conditions contribute to the positive correlation between $\delta^{13}C$ and productivity across the majority of sites. However, the two sites with the lowest productivity were those with the greatest $\delta^{13}C$ values, though in this case they were negatively affected by severe water stress. SMO is a very coarse textured site that received an annual average of 432 mm of precipitation over the three year growing period. Although UOS is a clay soil, it experienced extended periods without rain particularly in July 2007 [16.4 mm of precipitation in July as compared to a 30 yr-normal of 60.1 mm (Environment Canada, 2011)]. It is therefore reasonable to conclude that the poor productivity at these two sites is primarily a result of water stress due respectively to soil type and summer precipitation. Thus, while these two sites were limited by water stress, other sites were limited by other factors.

Lindroth and Båth (1999) measured willow water use efficiency at 6.3 g of dry matter produced per kg of water transpired; that is, when nutrient availability was not a factor. Given this information, they were then able to construct a simple model [3.1] to predict the upper limit of willow growth given the restraints of water limitations:

$$W_s = \tau(1 - c_l - c_\tau)P\omega \qquad [3.1]$$

Where W_s is the weight of dry matter produced in stems, τ is the water use efficiency, c_1 and c_τ are the proportion of mass in leaves and roots, P is precipitation and ω is the transpiration fraction of evapotranspiration. Applying this model and the estimates given for τ, c_1, c_τ and ω (derived from *S. viminalis*) to the total annual precipitation at the study sites yields a range in maximal productivity from 7.9 to 20.6 Mg ha^{-1} yr^{-1}, assuming similar water use efficiency for *S. purpurea* 'Hotel'. Production rates varied between 1.13 and 11.0 Mg ha^{-1} yr^{-1} dry weight which is much lower than the predicted maximum. However, considering that in this experiment the establishment year was included in productivity calculations (with the exception of GUE), the actual rate of growth in the third year of specific sites would have likely approached or exceeded the potential as calculated based on precipitation. For example, at LAK the maximum recorded productivity after three years was 21.8 Mg ha^{-1} whereas the predicted maximum for the third year is 7.9 Mg ha^{-1}. Because growth is known to be lower early in establishment than the eventual maximum (Volk et al., 2004), it is unreasonable that little more than one third of the growth occurred in the third year. Thus, water use efficiency likely exceeded that of 6.3 g kg^{-1} at this site. Therefore, *S. purpurea* 'Hotel' either has a slightly higher water use efficiency compared to *S. viminalis* or willow water use efficiency can rise with increasing scarcity up to some threshold as has been seen in *Populus* (Yin et al., 2005) and was noted from ^{13}C isotopic signatures in this study. For those sites with lower productivity and low water stress, this supports that factors other than precipitation, evapotranspiration and climate in general are negatively controlling the growth of these trees. As other limiting factors, i.e. nutrition, are removed productivity increases such that more water is used thereby increasing water stress and as a result water use efficiency.

Soil texture and topography can also have a large impact on water availability for plants. Coarse soils allow water to quickly drain such that even soils that receive adequate precipitation may not be able to retain enough moisture long enough to support high rates of plant growth, as per SMO. Likewise, topography can affect moisture availability by affecting water redistribution. However, this was likely not a factor as the study sites were mostly flat. Productivity of willow plantations early in their establishment stage has been reported as greatest in medium textured soils (Abrahamson et al., 2002; Mitchell et al., 1999). This is presumably because sandy soils

lack the water retention and nutrient availability required for willow growth and heavier clay soils are too difficult for young roots to penetrate. Indeed, productivity was negatively correlated with sand content ($r = -0.547$, $P = 0.001$) and positively correlated with silt content ($r = 0.715$, $P < 0.001$).

Mitchell et al. (1999) stated that willow requires soils with good drainage and that stagnating water will result in a decline in production. The results of this study indicate that where high clay content layers exist growth is hindered. However, this trend exists even where precipitation is low thus making the stagnating water argument improbable for these study sites. Likewise in the upper 20 cm in this study, willow productivity increases as both silt and clay content increases with the exception of soil with clay contents above 0.5 g g^{-1} (Figure 3.4a,b). At 20-40 cm depth, silt and sand are positively correlated with productivity at low concentrations and negatively at high concentrations while clay is weakly negatively correlated (Figure 3.4d,e,f). Thus, the ideal soil texture for the willow included in this study is fine (clay) close to the surface to increase nutrient availability but overlying a slightly coarser texture (clay loam) perhaps allowing for easier root penetration.

Drought is common on the Canadian prairies but it is not the only weather associated limiting factor. Extreme cold in winter months is particularly hard on young shoots and some winter kill was noted at the UOS site, and nearly caused plantation failure at the BIR site. At the latter site, extreme cold during the winter (minimum daily temperatures <-40°C) was blamed for killing most of the aboveground biomass. To restore plant health, the willows were coppiced to encourage growth from the still healthy base of the plants. This made it impossible to compare the rates of productivity at the BIR site with other plantations but it does highlight the potential of extreme weather to limit the productivity of a plantation. Therefore, while it is still important to look at typical rates of precipitation and the length of growing season, it may be more important to look at the probability of extreme weather when evaluating the suitability of a site for willow production.

3.5.2 Soil chemical composition

Soil chemistry was a strong indicator for the productivity of this willow clone. While soil texture appeared to have an impact on willow growth due to its influence on water retention, it seems that the acid-base status of the soil had a much greater control over willow productivity. The elemental composition of the soil can be grouped into categories of elements that co-vary and thus are similarly associated with productivity. As a whole, total Ca and total Mg were indicators of high productivity, while total Si was related to poor productivity (Figure 3.2). This indicates that soils containing calcite and dolomite were more conducive to willow growth than base-poor and acidic soils containing dominantly felsic minerals. This is confirmed by the prevalence of a positive total Ca term and negative total Al or total Fe term in multiple regression models. Dolomite and calcite weather faster than most Ca-rich minerals contained in felsic rocks (e.g. plagioclase, amphibole) and thus in a young landscape that was "recently" glaciated, a soil that contains Ca and Mg bearing minerals that are easily dissolved will be likely more productive. While I am unaware of studies showing such a relationship between total Ca and tree growth, a few studies with sugar maple indicated the benefits of total Ca-rich soils on growth variables, notably seedling survival and positive correlations with arbuscular mycorrhizal fungi (Juice et al., 2006; Kobe et al., 1995). This paper may therefore serve to show that total chemistry of the soil, which is not a common soil analysis, can be a reliable indicator of tree growth and that it can provide different information than the most commonly tested exchangeable cations (Thiffault et al., 2006).

Calcium and Mg-rich soils also create an environment that has an appropriate pH level which, in turn, is generally a good indicator for the suitability of a soil for plant growth as it affects cation exchange capacity of soils, soil microbial activity and nutrient availability for plants (Havlin et al., 2005). Soil pH was strongly (positively) correlated to total Ca ($r = 0.90$) and total Mg ($r = 0.95$). *S. purpurea* 'Hotel' grew best in soils with a pH of approximately 8.3 but growth declined sharply below 7.0. Anything below a pH of 6.0 showed very poor productivity. This is higher than the site recommendations by Abrahamson et al. (2002) of between pH of 5.5 and 8.0 and by Mitchell et al. (1999) of between pH of 6.0 and 7.0.

Soil C, due to its role in increasing soil fertility and moisture retention (Havlin et al., 2005), was positively correlated to productivity (Figure 3.3). Higher soil C is generally associated with higher rates of primary productivity and as such would suggest that the soils most capable of supporting high rates of willow biomass production will also be the same soils producing the greatest yields for agriculture [i.e. soils high in soil C (Christensen and Johnston, 1997)]. However, the strong affinity for total Ca and pH >8.0 exhibited by *S. purpurea* 'Hotel' may provide an exploitable niche within agricultural regions. This study confirms growth of willow in degraded soils or those low in soil C will have lower productivity. However, should tillage decrease with establishment of perennial willow, soil C would be expected to increase (Six et al., 2002), leading to a positive feedback of increased willow productivity and soil C over time.

3.5.3 Willow Foliar Nutrition

Although the ultimate goal of this study was to determine site quality from soils, foliar nutrition is a more direct measure of the status of the plants and as such was used for determining site quality. When examining the effects of nutritional concentration of willow as a predictor for productivity, it is important to take into consideration that two sites were limited by water stress. Without adequate water availability, the effect of nutrient deficiencies on growth is minimal. Thus, these sites were excluded from the dataset when trying to link foliar nutrients to tree growth. This assumes that water stress is not affecting any other site and that any nutrient deficiency has negligible effects on those plants experiencing water stress, no matter how severe the deficiency.

The two nutrients that had the strongest relationship with willow growth were N and K, not Ca and Mg (Figures 3.5 to 3.9). It is for this reason that I suspect that the acid-base status of the soil is a proxy for nutrient availability as a whole, not just for Ca and Mg. This also explains why exchangeable Ca and Mg were poor predictors of foliar concentrations. Nitrogen was positively correlated with productivity both in terms of total N in the soil as well as in the leaves (Figure 3.5). The highest foliar N concentrations in the dataset were approximately 30 mg N g^{-1} which falls in the range of optimum concentrations (22 to 35 mg g^{-1} depending on species)

reported by Simon et al. (1990) and Kopinga and van den Burg (1995). The relationship was linear and did not flatten out at the higher foliar levels, demonstrating the high requirements of willow for N for maximum growth. The vast majority of total soil N is organic N but the inorganic N fraction that is readily available for plant uptake. However, there were negative correlations between soil inorganic N and willow growth (Table 3.3). This difference can be explained as a change in N cycling and an increased depletion of inorganic N by faster growing trees (Chapter 4). The same effect was observed by Moukoumi et al. (2012) in pure willow plantations in Saskatchewan as well as intercroppings with the N-fixing caragana shrub.

The effect of K nutrition on productivity is also shown by the strong relationship between foliar K, exchangeable soil K and growth (Figure 3.7). Below a leaf concentration of between 8.0 and 10.0 mg K g^{-1}, growth is severely impaired. This corresponds well with the threshold value for K deficiencies reported in the literature (Mengel, 2007). Above this value, *S. purpurea* 'Hotel' responded strongly to increasing concentrations of foliar K. Potassium also appeared sufficient at soil exchangeable concentrations of 0.5 $cmol_c$ kg^{-1}. No upper bounds where excess K was taken up were detected in this study.

Foliar Mg was found to be a good indicator of willow growth only after the aforementioned factors were first taken into account, specifically water stress and deficiencies in K and N. At sites where Mg was high but K and/or N was low, the limiting effects of K and N deficiencies far outweighed any benefit provided by the additional Mg (Figure 3.7). At LAK, POR, and PIC, however, there was a positive response to increasing foliar concentrations at least to 4.0 mg g^{-1} (Figure 3.7a), which indicates the >3.0 mg g^{-1} value for optimal nutrition reported by Kopinga and van den Burg (1995) is low for *S. purpurea* 'Hotel'. Thus Mg will increase in importance as deficiencies in water, N and K are alleviated. However, there was no clear relationship between exchangeable soil Mg and either foliar concentration or productivity, making prediction of Mg soil requirements for willow difficult.

Foliar Ca was also found to be correlated with productivity (Figure 3.8a) but its effects as a nutrient is inseparable from its effect as the key buffer for pH and therefore it is difficult to discern Ca nutrition from soil analyses. While the full causation of soil Ca on productivity is

unclear, the low productivity below 18-20 $cmol_c\ kg^{-1}$ exchangeable Ca seen in Figure 3.9b indicates caution should be given to establishing willow on sites with low exchangeable Ca.

From 0.4–1.2 mg g^{-1} H_2SO_4 leached soil P, covering much of the range seen in this study, the foliar P concentration remained relatively stable near 2.0 mg P g^{-1} (Figure 3.6c). This is the optimum nutrition of willow leaves as reported by Simon et al. (1990). Therefore the lack of relationship between foliar and soil P and willow growth (Figure 3.6a, b) is due to the ability of the willow to effectively regulate P uptake within the range of conditions experienced in this study. Concentrations of foliar P varied from the optimum only where leachable soil P was at the extremes of the range of concentrations seen within this study. Interestingly, the relationship between soil P and foliar P suggest that the acid leach is a good proxy for available soil P in SRIC of willow (Figure 3.6c).

3.6 Conclusions

From this study, it appears that soil properties are the most important for estimating *S. purpurea* 'Hotel' productivity in the Canadian prairies and southern Ontario. Prediction of willow site quality is possible given mainly soil data despite a large climatic gradient. Characteristics which affect water retention and nutrient availability are the most useful for predicting willow growth. The acid-base status of the soil as dictated by bulk elemental composition, in particular the presence of Ca, was found to be the dominant control ($r^2 = 0.87$, $p < 0.001$ total Ca vs. productivity) despite large regional differences in climate. From a nutritional standpoint, sites with more available N ($r^2 = 0.45$, $p < 0.001$ foliar N vs. productivity with adequate moisture) and K ($r^2 = 0.51$, $p < 0.001$ foliar K vs. productivity) also had greater growth. Water availability was found to be limiting productivity only at the two lowest productivity prairie sites but here moisture deficiencies overrode nutritional deficiencies. Thus, each site experiences its own unique combination of limiting factors: severe water stress > N and K deficiencies > Mg and P deficiencies. It is therefore expected that as one limitation is removed through selection of optimal sites, another becomes dominantly important. Therefore, productivity of *S. purpurea* 'Hotel' can be explained by Liebig's Law of Minimum as stated by

Taylor (1934) such that growth is limited largely by soil variables but that extreme environmental conditions can become limiting even though climate is not.

Preface to Chapter 4

Willow is known to be both water and nutrient demanding particularly when planted at high densities. Therefore it is important to examine the effects of this high demand in order to understand the ability of soils to maintain high rates of productivity. Chapter 4 compares various soil nutrient pools and soil C in willow plantations to that of reference sites. The effects of plantation establishment are discussed with particular attention given to nutrients in demand as highlighted in Chapter 3. Whereas Chapter 3 identified the site requirements for high rates of productivity, Chapter 4 introduces willow grown as SRIC as a feedback into the long term productivity of a given site.

4. EFFECTS OF FAST GROWING WILLOW PLANTATIONS ON SOIL C AND NUTRIENT AVAILABILITY

4.1 Abstract

Willow (*Salix* spp.) is currently being researched as a source of biomass energy in Canada. However, it is not certain whether afforestation with willow plantations will enhance or diminish nutrient availability and soil C storage. Trees are known to have pronounced effects on biologically mediated nutrient cycling processes which can increase nutrient availability. However, willow are known to be nutrient demanding. In this chapter, the net effect of plantation establishment is examined at nine sites across the prairie and southern Ontario regions of Canada. Nitrogen, P, K, Ca and C content of soils and harvestable biomass were compared between willow plantations and paired reference sites, at the end of the first three year rotation. Soils were depleted in inorganic N (-3.12 μg N g^{-1}, $P < 0.10$) and exchangeable K (-0.11 $cmol_c$ kg^{-1}, $P < 0.10$). Changes to acid leachable P (-0.03 mg g^{-1}, $P < 0.10$) and total C (-2.22 mg g^{-1}, $P < 0.05$) were related to the previous land use. Calcium content was not found to be consistently different from reference sites. Depletion of soil N, K, P and C was exacerbated by greater growth for N and K, diminished by greater growth for P and inconclusive as to the effects of productivity for maintaining C.

4.2 Introduction

There is growing interest for intensive (agro)forestry in Canada. Willow (*Salix* spp.) is one genus with potential to maintain high rates of productivity despite climatic limitations (Kopp et al., 2001a). Several willow species can be found growing across Canada, typically associated with low lying areas (Richards and Fung, 1969). Because of its hardiness, fast growth rate, and ability to regrow vegetatively, willow is ideally suited to short rotation intensive culture (SRIC), often in the context of energy production (Volk et al., 2004).

Although tree growth has been shown to respond favorably to fertilization (Adegbidi et al., 2001; Labrecque et al., 1998), trees are most frequently forced to be resourceful in nutrient acquisition (Bauhus and Messier, 1999; Fox, 2000). This is made possible by the trees' ability to

exploit nutrient pools through an extensive root system and to recycle nutrients efficiently. The introduction of trees in agricultural landscapes frequently has dramatic effects on soil nutrient availability by: (1) accessing resources from deeper within the soil profile, (2) augmenting soil mineral weathering by producing acidic exudates (roots) that act as chelators, (3) changing hydrologic conditions and increasing evapotranspiration to decrease leaching, (4) storing nutrients within their biomass and their leaves which are then returned to the soil via litterfall or other pathways, (5) filtering of dry particulates by the forest canopy, and (6) altering pH and suppressing competition (Attiwill and Adams, 1993; Binkley and Giardina, 1998; Augusto et al., 2002).

Nutrient cycling in SRIC, however, is not as efficient as in a natural forest (Augusto et al., 2002). This is because fast growing tree species such as *Salix* spp. use high levels of nutrients (Simon et al., 1990; Kopinga and van den Burg, 1995; Cornelissen et al., 1997; Mitchell et al., 1999) and some of the nutrients the trees take up will be lost from the soils for future use when the biomass is extracted (Adegbidi et al., 2001; Augusto et al., 2002). However, some of the biologically mediated processes identified above may increase nutrient sequestration and availability and therefore, rapid tree growth does not always result at the expense of soil nutrients. In fact, some of these processes may add nutrients to the soil system and may even be enhanced via increased growth (Bélanger et al., 2004). For example, the larger amount of leaf surface area of a faster growing tree will filter out more particulates that are added to the soil as they are washed off by rain (Augusto et al., 2002). Increased root growth and acidic exudate production can augment the release of nutrients from parent material mineral weathering (Augusto et al., 2002). Additionally, when a site is nutrient poor, trees increase their root to shoot ratio (Ericsson, 1981) which may result in enhanced weathering of parent materials due to increased organic acid production (Bormann et al., 1974; Quideau et al., 1996). As well as these additions, it may be that while larger trees are taking up more nutrients, they are also increasing the recycling of these nutrients through decreased leaching. Greater evapotranspiration and a more complex/developed root system reduces the amount of nutrient-rich waters percolating and leaving the soil system (Augusto et al., 2002; Bélanger et al., 2004).

To further complicate the issue, changes to soil nutrient pools, soil C and soil pH all depend on the previous land use (Hobbie, 1992). For each soil property, there are a number of ways that plantation establishment can have an effect. Firstly, plantation establishment can have a unidirectional effect. This would be the case if willow always caused a decrease in available N as it is exported with biomass until it is no longer economically viable to produce willow on that site. The rate of depletion will depend on the initial concentration in the soil and the affinity of willow for that nutrient. In the case of C, in other agroforestry systems, the literature describes a different way for change to occur. For each soil and climate, there is roughly a steady state equilibrium of soil C depending on land use (Guo and Gifford, 2002; Olszewska and Smal, 2008; Laganière et al. 2010). Agroforestry systems tend to have higher soil C than annual crops but lower than pastures (Guo and Gifford, 2002). Land use change initiates a gradual change in soil C to the steady state associated with the particular land use (Guo and Gifford, 2002). Therefore, willow plantation establishment on pasture may lead to a decrease in soil C but establishment on annual cropland may increase soil C.

Thus, questions arise as to the impact of willow plantations on soils. Do willow plantations provide the right conditions for enhanced nutrient and C sequestration? Are the nutrients removed with biomass irreplaceable without human intervention? Investigations of the nutritional status of willow plantations needs to be extended from measurement of nutrient export rates to include the effect of land use change on soil nutrient pools. We also need to know the nature of these changes. This information is crucial for determining the total effect of cultivating willow and the requirements of the system for any inputs and supplements both during and after production. Therefore, the objectives of this study are to measure nutrient export and quantify differences in soil nutrient and C stocks between plantation and reference soils as result of willow plantation establishment and first rotation harvest across a wide range of soils and land-use in Canada. The hypothesis that willow plantation establishment maintains soil nutrients is tested. The nature and direction of the changes are also examined in order to understand those pools that may be of concern for maintaining future productivity.

4.3 Materials and Methods

4.3.1 Experimental design

Nine sites across the Canadian prairies and extending into eastern Canada were included in this study representing a wide variety of soil types and previous land uses (Table 4.1).

Table 4.1: Site names, locations, history, soil texture, soil pH, precipitation and harvested biomass summary data for nine *S. purpurea* 'Hotel' plantations included in this study.

Site (ID)	Geographic Coordinates	Site History	Soil Texture	Soil pH (SE)	Mean Annual Precipitation (SE)†		Year Planted	Year Sampled	Harvested Biomass (SE)
					30 Year Mean	Production Years			
					—— mm ——				Mg ha^{-1}
Ellerslie Research Station, AB (ELL)	53°25'N 113°31'W	Cereal crops	Silty Clay Loam	6.0 (0.1)	444 (14)	404 (43)	2005	2007	N/A ¶
Smoky Lake, AB (SMO)	54°07'N 112°24'W	Tree nursery‡	Sandy Loam	5.1 (0.1)	432 (13)	412 (27)	2005	2007	5.4 (0.1)
Lakeshore Tree Nursery, SK (LAK)	52°00'N 106°45'W	Pasture	Silty Clay Loam	8.3 (0.0)	349 (16)	454 (53)	2005	2007	18.4 (1.2)
University of Saskatchewan, SK (UOS)	52°07'N 106°36'W	Cereal crops	Clay	6.6 (0.1)	364 (17)	382 (74)	2006	2008	5.3 (0.8)
Portage la Prairie, MB (POR)	49°57'N 98°10'W	Vegetables	Silt Loam	8.3 (0.0)	537 (17)	523 (88)	2005	2007	22.8 (3.2)
Bird's Hill, MB (BIR)	50°00'N 97°00'W	Pasture	Heavy Clay	8.3 (0.1)	560 (21)	538 (91)	2005	2007	N/A ¶
Sault Ste Marie, ON (SSM)	46°32'N 84°24'W	Managed forest	Sandy Clay Loam	5.4 (0.1)	914 (26)	826 (56)	2006	2008	6.1 (0.3)
Guelph, ON (GUE)	43°33'N 80°13'W	Turf grass	Clay Loam	6.9 (0.0)	894 (26)	971 (143)	2005§	2008	9.5 (2.5)
Pickering, ON (PIC)	44°00'N 79°01'W	Idle / Failed plantation	Clay	6.8 (0.2)	887 (18)	948 (131)	2006	2008	14.0 (2.1)

† Modeled using BioSIM.
‡ Unable to find ideal optimal match to previous land use; used surrounding forest.
§ Coppiced after first year; three years of aboveground biomass.
¶ Harvested previously such that comparable data is unavailable.

Unrooted cuttings of the 'Hotel' clone of *S. purpurea* were planted in triple rows at a spacing of 60 cm within rows and 200 cm between rows. Plantations were established in 2005 and 2006 and sampled in the third (i.e. final) year of the first rotation (i.e. 2007 and 2008) with soil samples being collected in August and stems in October/November. Whereas willow bioenergy plantations are typically coppiced at the end of the first year to promote branching, the sites included in this study—with the exception of GUE—were not coppiced at the end of the first year. GUE was coppiced after the first year and then allowed to complete a three year rotation such that at this site the land use change had occurred four years previous as opposed to three years for the other sites. At each site, five 3.2 m x 1.8 m plots from within the plantation were sampled together with five reference plots from the adjoining area. The reference sites were chosen be characteristic of the land-use at the time the plantations were established. Likewise, the reference sites were selected on soils comparable to those in the plantations. Within each 3.2 m x 1.8 m plot, soil cores (n = 3) were collected at 0-20 and 20-40 cm increments; one core was taken from the centre of the triple row, one from the edge of the triple row and one from halfway between triple rows and analyzed separately. Stems from the trees originating within each plot were harvested and weighed in the field and converted to dry weight by reweighing representative sub-samples (Ens et al., 2009). Stems were weighed and collected at only seven of the nine sites as two sites had previously been coppiced in the spring.

4.3.2 Laboratory analyses

Nutrient removal rates were calculated from the mass of harvested stems (Ens et al., 2009) and their concentrations of N, P, K, Ca, Mg, and Na. Concentrations of total N (and C) were obtained during C isotope analysis (Chapter 3) using a RoboPrep Sample Converter interfaced with a TracerMass Stable Isotope Detector (Europa Scientific, Crewe, UK). Phosphorus, K, Ca, Mg and Na were extracted using concentrated H_2SO_4—H_2O_2 digests (Kalra and Maynard, 1999). Concentrations of PO_4 were measured colorimetrically using a SmartChem instrument (Mandel Scientific Company Inc., Guelph, ON). Potassium, Ca, Mg, Na were determined by atomic emission (K, Na) and absorption (Ca, Mg, Fe, Al) spectroscopy (SpectraAA 220, Varian Analytical Instruments).

Differences in soil chemistry compared soil C, NO_3, NH_4, P, K, Ca, Mg, Na, Fe, Al, pH between the plantation and reference plots. Total soil C was determined by infrared detection on a LECO C Analyzer at 1100°C. Nitrate and NH_4 ions were extracted using 2.0 *M* KCl and analyzed on a Technicon AutoAnalyzer (Technicon Instruments Corporation, Tarrytown, NY). Total P was extracted by H_2SO_4-H_2O_2 digests modified from Kalra and Maynard (1999) and again measured colorimetrically with the SmartChem instrument. Exchangeable K, Ca, Mg, Na, Fe, and Al were extracted via a 0.1 *M* $BaCl_2$ solution (Hendershot et al., 2008) and analyzed by atomic emission (K, Na) or absorption (Ca, Mg, Fe, Al) spectroscopy as with plant digests. Soil pH was measured in a 2:1 water to soil ratio on a weight basis.

4.3.3 Statistical analyses

Differences in soil properties between plantation and reference soils were detected at individual sites using the Student's t-test. Effects of land use change across all sites were tested using paired t-tests. Significant ($P < 0.10$ was used to guard against type II errors) effects were further investigated by graphical interpretation of differences. Logarithmic transformation was performed as necessary to assure normality in the residuals and equal variance. Statistical analyses were conducted in CoStat Ver. 6.1 (CoHort Software, Monterey, CA).

The nature and direction of change was examined graphically for various soil properties. The nine sites included in this study represent a wide range of physiographic conditions which affect the magnitude, direction and nature of soil chemical changes with plantation establishment. The net effect on soil chemistry depends upon the initial condition of the soil (e.g. initial concentration of K), the exchange capacity of the soil, and the ability of willow to alter the soil (i.e. by uptake, weathering, sequestration, etc.). By using the reference sites as a proxy for initial concentration, it is possible to represent change from plantation establishment as a function of initial conditions.

The analysis thus makes the assumption that the reference sites are at a steady state and accurately reflect the conditions of the plantation immediately prior to establishment. After making these assumptions, the values can be applied for different soil properties from the reference soils as the initial concentration for both the reference site and the plantation.

Therefore, a 1:1 line can be produced when initial concentration of the reference site is on the x-axis and concentration after the first rotation is on the y-axis. This is because the x and y values for the reference site are the same value according to the assumption of steady state. Linear regression of the reference (x-axis) and plantation (y-axis) yields a line that can be visually and statistically compared to the 1:1 line (i.e. the reference site), with any deviation from the 1:1 line indicating a change resulting from plantation establishment. Differences in slope and intercept also describe the nature of the change. A slope of <1.0 describes a negative feedback, meaning that there is either more depletion or less accumulation at higher initial concentrations. A slope of >1.0 indicates a positive feedback where soils with high initial concentrations increase more or are able to better buffer changes than those with lower initial concentrations. The intersection point of the two lines occurs at their steady state concentration. A slope of one indicates that any change is independent of initial conditions. By examining the dataset as a whole, the effects of within site variability on the accuracy of the reference site as an estimate of the true initial conditions is minimized.

4.4 Results

Sites were widely variable in soil texture (0.03-0.74 g g^{-1} sand, 0-20 cm) and cation exchange capacity (2.2-53.2 $cmol_c$ kg^{-1}, 0-20 cm). Total C , inorganic N and leachable P were consequently also highly variable (21.2-68.8 mg g^{-1}, 1.6-14.1, µg g^{-1}, 0.62-1.15 mg g^{-1}, respectively at 0-20 cm). This substantial range in conditions means that there is a large difference in the potential for willow to affect soil chemistry and confirms the need for statistical analyses to be conservative for type II errors (i.e. $P < 0.10$).

Table 4.2: Differences between *S. purpurea* 'Hotel' plantation and reference soils calculated by paired t-test for nine sites (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC).

Variable	Change at 0-20 cm	Change at 20-40 cm
Total C (mg g^{-1})	-2.22*	-0.43
Inorganic N (µg N g^{-1})	-3.12†	-2.60†
NH_4 (µg N g^{-1})	-0.95†	-1.38†
NO_3 (µg N g^{-1})	-2.17†	-1.22†
Total P (mg g^{-1})	-0.03†	-0.02†
Exchangeable cations		
K ($cmol_c$ kg^{-1})	-0.11†	-0.10†
Ca ($cmol_c$ kg^{-1})	-2.10*	-2.57*
Mg ($cmol_c$ kg^{-1})	0.30	-0.11
Na ($cmol_c$ kg^{-1})	-0.05†	0.04†

*Significant at 0.05 probability level.
†Significant at 0.10 probability level.

The results indicate soil C in the upper 20 cm of soil decreased consistently (-2.22 mg g^{-1}, $P < 0.05$) across sites regardless of the initial concentrations (Figure 4.1). At lower depths there was much more variability in soil C but overall soil C remained stable at 20-40 cm (Figure 4.1, Table 4.2).

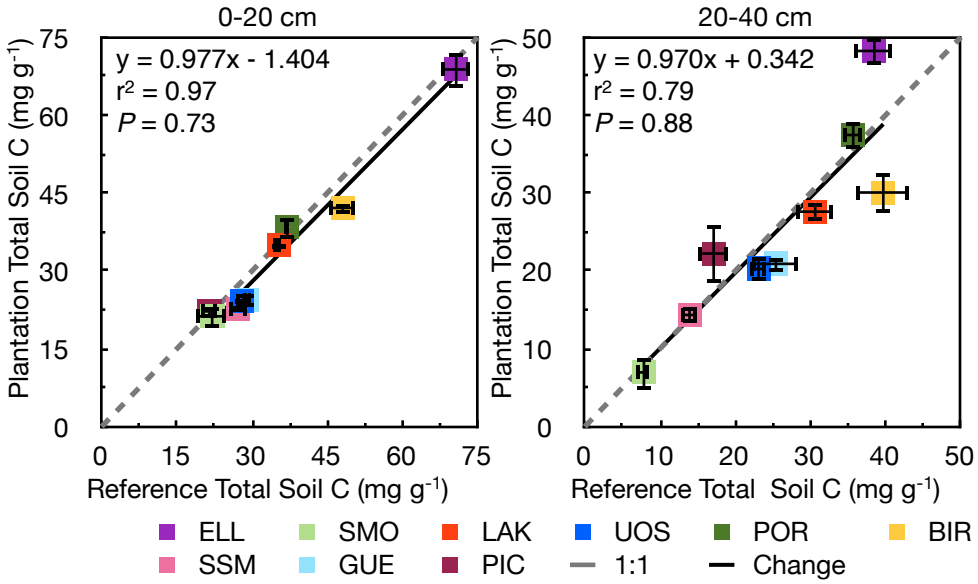

Figure 4.1: Initial concentration of total soil C, as estimated from reference sites, versus total soil C concentration in plantations after the first rotation of nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Error bars represent standard error.

At sites where inorganic N concentrations was initially high, both NO_3 and NH_4 ions were depleted but there was a much more pronounced effect on NO_3 (slope = -0.065, NO_3; slope = 0.646, NH_4; Figure 4.2). Inorganic N and productivity were negatively correlated (total inorganic N, r = -0.88; NO_3, r = -0.87; NH_4, r = -0.31).

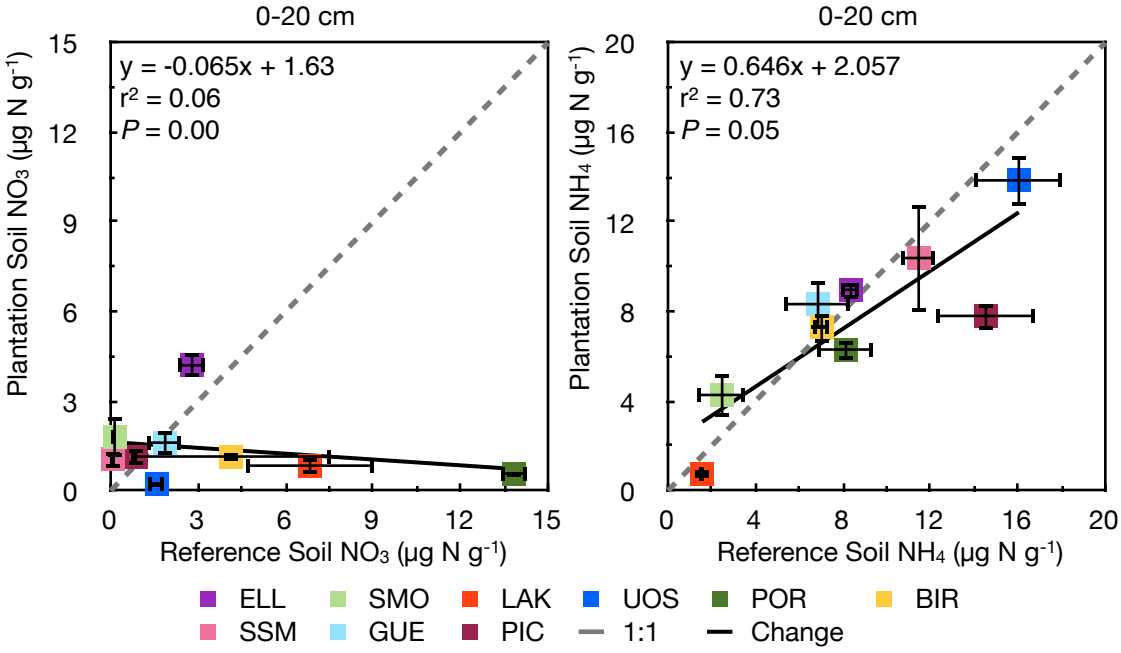

Figure 4.2: Initial concentration of NO_3 and NH_4 as estimated from reference sites, versus NO_3 and NH_4 in plantations after the first rotation of nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Error bars represent standard error.

Phosphorus was found to be depleted with increasing initial concentrations (slope = 0.737, 0-20 cm; slope = 0.769, 20-40 cm; Figure 4.3). At lower initial concentrations there was a tendency towards more stable or increasing phosphorous stocks. The greatest losses occurred on soils high in P but with low rates of productivity.

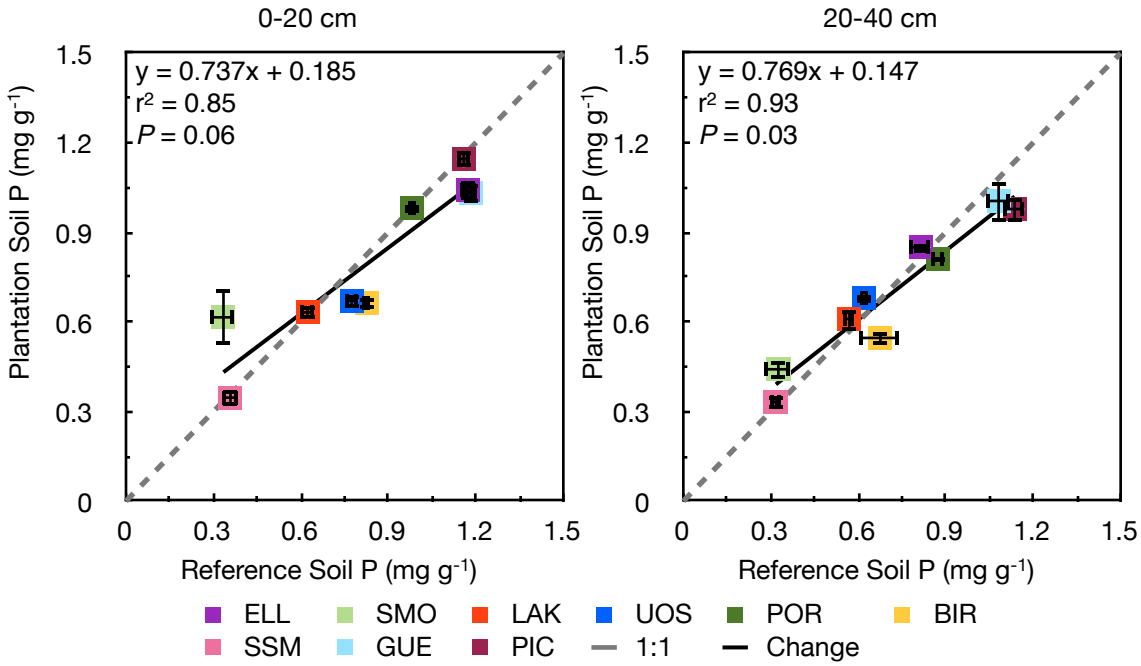

Figure 4.3: Initial concentration of acid leachable P, as estimated from reference sites, versus acid leachable P concentration in plantations after the first rotation of nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Error bars represent standard error.

Potassium was found to be depleted as a result of plantation establishment ($P < 0.10$). Change in K was both related to productivity (r = -0.78, $P = 0.07$) and initial concentration of K (Figure 4.4). The greatest losses occurred on soil rich in exchangeable K and with high rates of productivity (Figure 4.5).

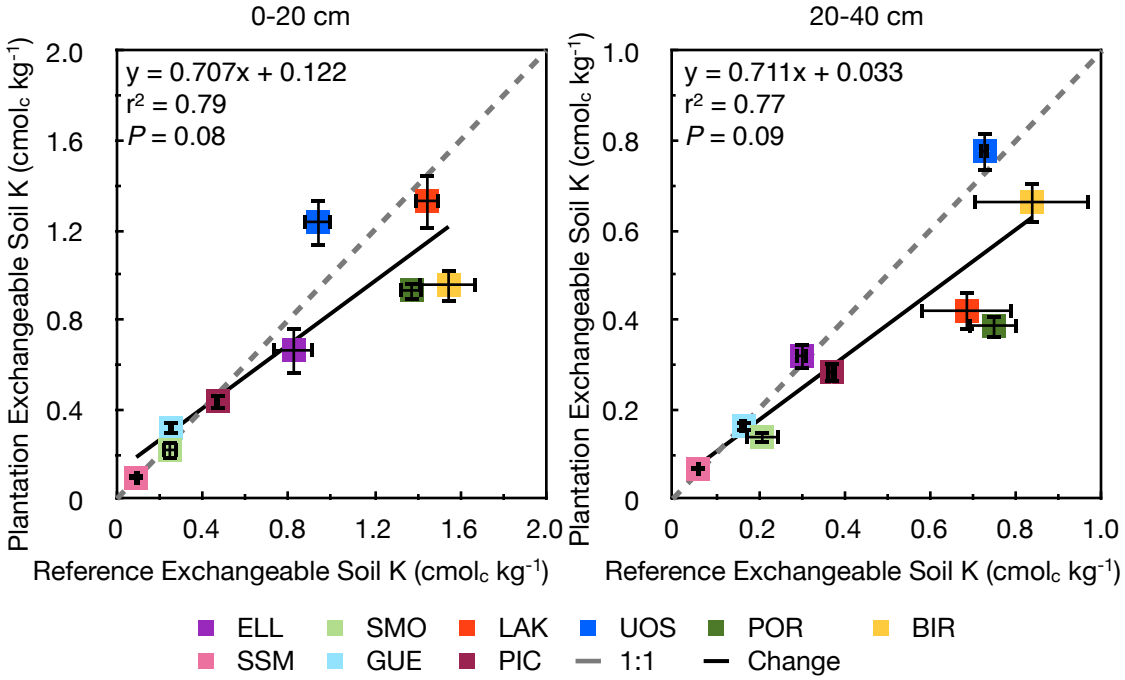

Figure 4.4: Initial concentration of exchangeable K, as estimated from reference sites, versus exchangeable K concentration in plantations after the first rotation of nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Error bars represent standard error.

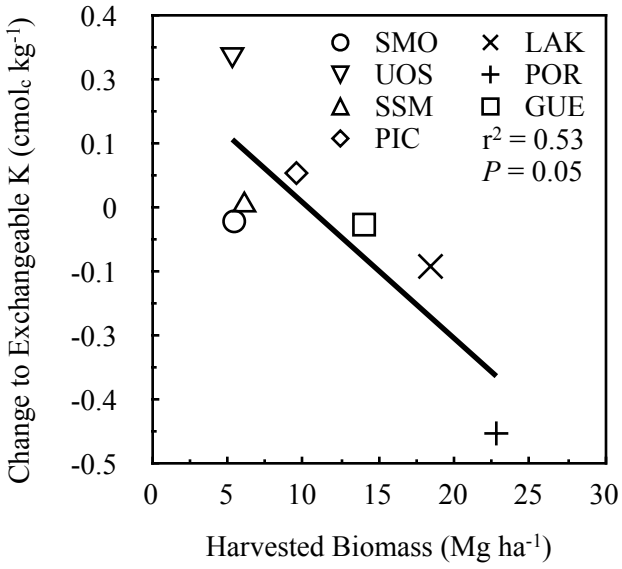

Figure 4.5: Harvested biomass (Mg ha^{-1}) vs. change in exchangeable K concentration (cmolc kg^{-1}) after the first rotation of seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC).

Paired t-tests indicated that there was a significant and substantial loss of exchangeable Ca (Table 4.2). However, closer examination with site by site t-tests revealed that this result for the 0-20 cm depth was forced by a single site, i.e., LAK, and that no other site shared in this loss (Figure 4.6). LAK was thus an outlier and there was no actual loss of Ca with plantation establishment.

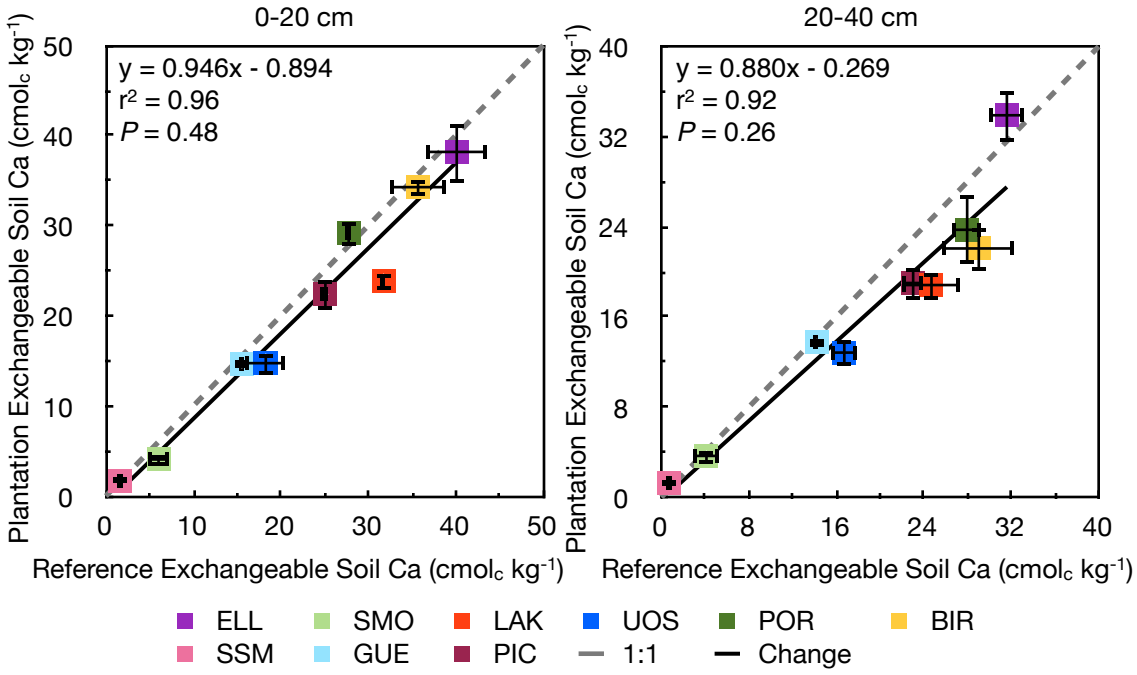

Figure 4.6: Initial concentration of exchangeable Ca, as estimated from reference sites, versus exchangeable Ca concentration in plantations after the first rotation of nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Error bars represent standard error.

There was no relationship between Mg and plantation establishment. Paired t-tests indicated a depletion of Na in top 20 cm (-0.05 $cmol_c$ kg^{-1}, $P < 0.10$) which was matched by an increase at 20-40 cm (0.04 $cmol_c$ kg^{-1}, $P < 0.10$) (Figure 4.7).

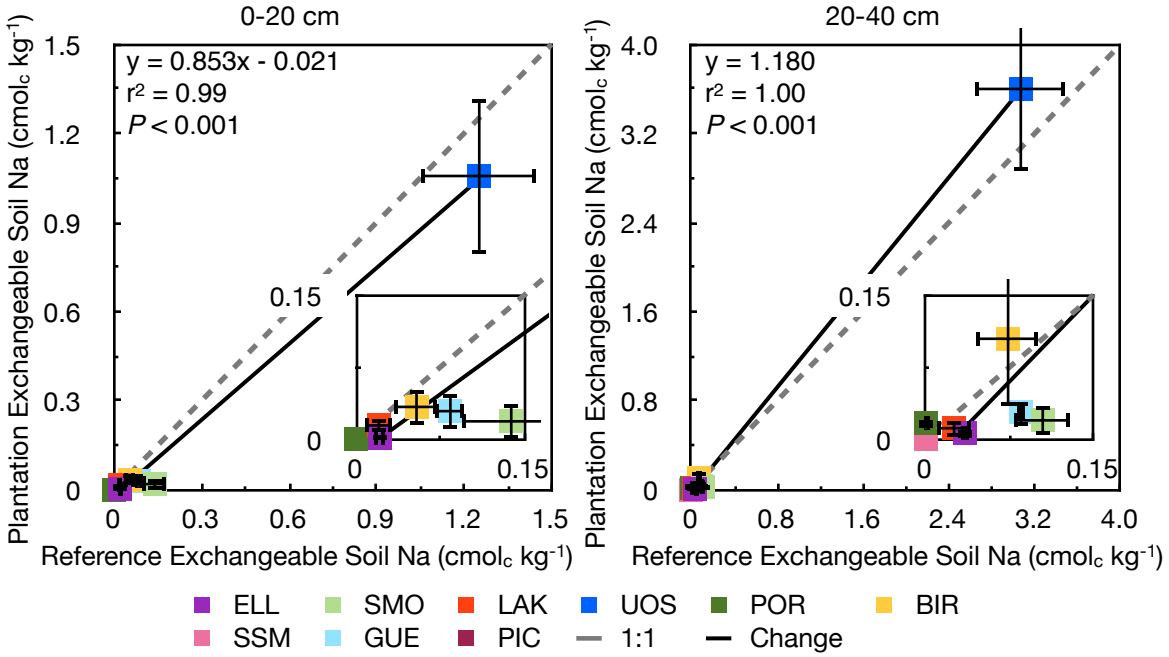

Figure 4.7: Initial concentration of exchangeable Na, as estimated from reference sites, versus exchangeable Na concentration in plantations after the first rotation of nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC). Inset shows expanded view of low values. Error bars represent standard error.

Concentrations of nutrients in *S. purpurea* 'Hotel' (mean of 6.4, 0.87, 2.1, 5.2 and 0.75 mg g^{-1} N, P, K, Ca and Mg) were found to be within the expected range for *Salix* spp., with the exception of K, which was lower in this study (mean of 2.1 mg g^{-1} vs. expected range of 2.9-3.4 mg g^{-1}) (Labrecque et al., 1998; Jug et al., 1999; Adegbidi et al., 2001). Nutrient export was limited primarily by rate of productivity (Table 4.3).

Table 4.3: Nutrient export as determined from total nutrient content (N, P, K, Ca, Mg) of harvested biomass by *S. purpurea* 'Hotel' in seven plantations expressed on an annual basis.

Site	Productivity	Export				
		N	P	K	Ca	Mg
	Mg ha^{-1} yr^{-1}	kg ha^{-1} yr^{-1}				
SMO	1.8	13.2	1.89	2.79	9.0	1.22
LAK	6.1	41.8	4.15	1.95	30.3	4.77
UOS	1.8	12.6	1.69	3.18	7.2	2.36
POR	7.6	34.4	5.12	1.78	39.8	5.11
SSM	2.0	13.8	1.31	1.52	11.7	1.46
PIC	4.7	33.3	5.08	2.06	24.5	1.99
GUE	3.2	17.1	3.13	1.61	18.9	1.98
Mean	3.9	23.8	3.19	2.13	20.2	2.70

4.5 Discussion

4.5.1 Soil Carbon

Paul et al. (2002) described an initial soil C loss associated with afforestation. This is however a reversible one-time event associated with the disturbance of soil and vegetative communities and the time it takes for primary productivity (and in turn, litter input) to recover (3-35 years) before a gradual increase in soil C (Paul et al., 2002). The data from this study supports the expected outcome that there is an initial loss of soil C with plantation establishment. Soil organic C in the upper 20 cm of soil showed a consistent loss across sites, regardless of the initial concentrations (Figure 4.1). However, at lower depths, soil C remained stable (Figure 4.1, Table 4.2). Trees tend to accumulate C at the surface from litterfall and have a greater proportion of roots deeper in the soil profile compared to grasses (Jobbágy and Jackson, 2000). Consequently, afforestation has been shown to redistribute soil C both deeper in the soil profile and in the forest floor such that soil C levels in the upper mineral soil may be depleted but total soil C stocks remain unchanged (Paul et al., 2002; Pinno and Bélanger, 2008). As such, it is expected that soil C will rise in subsequent years.

If the accumulation of coarse woody roots was included in the C budget, however, one would probably realize that planting trees has benefits on overall below ground C sequestration

(Laganière et al., 2010). Extensive research has been conducted into the effect of C storage with land use change. For any given soil, the stable concentration of soil C is generally greatest for pastures and forests, followed by plantations and finally cultivated soils (Guo and Gifford, 2002). This relationship can be explained by the allocation of C in grasses compared to trees. Grasses produce copious fine roots which turnover rapidly, adding C to the soil. Trees, on the other hand, allocate a greater proportion of their C to aboveground stems. They also produce larger, woodier and longer lived roots which do not add the same supply of organic inputs to the soil. Therefore, soils in treed systems tend to have less soil C than do grasses in similar physiographic regions although total system C can be much greater and large amounts of C are stored below ground as woody roots (Laganière et al., 2010).

It is also worth noting that the loss in soil C was greatest at the plantations that were established on sites previously vegetated by perennial grasses or trees (SMO, LAK, BIR, SSM, GUE, POR). At these sites, reviews by Guo and Gifford (2002) and Laganière et al. (2010) suggest that soil C may not fully recover within the 20-30 year life expectancy of a willow plantation. The recovery process will be greatly affected by the continued management of plantations as with traditional agriculture on grassland C stocks. Tillage is known to enhance microbial decomposition thereby decreasing soil C through the turning of soil and the breakup of soil aggregates (Six et al., 2002). Therefore, the amount of C stored in a plantation soil is greatest when soil disturbance is minimized as with no-till farming (Turner and Lambert, 2000). This is an important consideration as, depending on management strategies, willow plantations may receive far more frequent disturbance than more typical plantations, owing to their short rotation length. Tilled sites also tend to lack the diversity of macrofauna, including earthworms, that enhance the formation of stable aggregates (Zou and Bashkin, 1998). Controlling the understory (competing) plants also removes an important C source (Woods et al., 1992).

4.5.2 Nitrogen

The decline in NO_3 and NH_4 can be explained as an increase in competition for available N as willow trees are known to be highly demanding of N, especially when they are planted at very high densities as with SRIC (Simon et al., 1990). Greater tree growth (and in turn N uptake) leads to more pronounced depletion of soil available N as evidenced by negative correlation

between net change of inorganic N and productivity. This indicates that the rate of uptake of N by willow exceeds the maximum rate of mineralization.

The results could be viewed as being positive in terms of the environmental impact−the depletion of the soil in N results in less NO_3 leaching and less potential for the release of nitrous oxide from denitrification (Aronsson and Bergström, 2001). However, it also indicates that the willow trees are N demanding and will likely require amendments with synthetic N fertilizers to enhance or maintain productivity unless alternative less energy intensive systems are developed [e.g. suitable N-fixing companion species in SRIC of willow (Moukoumi et al., 2012)]. The use of N fertilizers within bioenergy plantations will greatly depreciate the energy ratio and greenhouse gas balance.

4.5.3 Phosphorus

Unlike many studies that showed an increase in available P with afforestation [see review by Chen et al. (2008)] there was a decrease at higher initial P concentrations detected in this study. However, it must be noted that part of this detectable change is due to an inadequate reference match at the SMO site. At SMO, P in the willow plantation was compared to the surrounding forest. The plantation had received P fertilizers in its recent history, thereby falsely indicating a willow induced rise in P with establishment. Thus, when SMO was excluded there was an overall 0.07 mg P g^{-1} soil loss ($P < 0.01$), four sites being depleted in P (ELL, UOS, BIR, GUE; $P < 0.05$) and four sites being unchanged (LAK, POR, SSM, PIC; $P < 0.05$).

Decreases in the organic fraction of soil P have been reported with site disturbance (Bormann et al., 1974) and afforestation (Chen et al., 2000). The decline in (mineral) soil P is apparently largely a result of the migration of soil P into the trees (leaves) through uptake by the roots and then translocated back to the soil as litterfall to form the forest floor−the inclusion of the newly created forest floor into the P budget can easily account for the losses in the mineral soil (Chen et al., 2000). However, the magnitude of the decrease at UOS and GUE was far greater than that of the estimated uptake, even if a generous assumption that only 20% of total P uptake was in harvestable stems (e.g. 10.4 and 17.9 mg P kg^{-1} soil uptake *vs.* 103 and 150 mg P kg^{-1} soil decrease respectively). The establishment phase of the plantation could well be

associated with increased losses of P, as degradation of organic matter from the previous land use continues but trees are still too small to recycle the released P (Bormann et al., 1974). Indeed, UOS and GUE had poorer rates of productivity while ELL and BIR had been coppiced recently, thereby maintaining a certain level of disturbance. On the other hand, the three sites with the fastest growth rates (LAK, POR, PIC) had no change in total P. Productivity at SSM was low but so too was the initial P content of the soil such that there was no change despite low rates of uptake.

Therefore, the results show that P is being efficiently cycled within plantations where P availability is matched by sufficiently high productivity. Substantial losses, during establishment, are most likely only to occur when P availability is high but productivity is low such as conversion from P fertilized crops when willow growth is limited by factors other than P (Ellert and Gregorich, 1996).

4.5.4 Potassium

Where soils are high in K and support high yields, K was depleted in the soils (Figure 4.4, Table 4.2). Certainly, harvestable stems do not contain all of the K sequestered by the willow but the detected changes are greater than can be explained by uptake alone (e.g., 89.3 mg K g^{-1} soil uptake *vs*. 173 mg K g^{-1} soil loss at POR assuming 20% of uptake contained in harvestable stems). Site disturbance and the subsequent release of immobilized K has previously been shown to dramatically increase export of dissolved K (Likens et al., 1994). Additionally, losses in soil C will have resulted in fewer exchange sites to which K can adsorb [mean cation exchange capacity (CEC) decreased 1.97 $cmol_c$ kg^{-1} in my study]. Thus, K, being readily soluble, is affected by site disturbance and changes in water use associated with devegetation and reestablishment of tree species (Likens et al., 1994). Decreased water use, early in the plantation's life-cycle, allows for increased drainage thereby multiplying losses of soil solution K and eventually exchangeable K.

It is important to note that K has been shown to remain in this enhanced state of mobility several years after disturbance and revegetation occurs (Likens et al., 1994). This allows for the continuation of leaching even while uptake increases as trees grow and as such depletion is

exacerbated by increasing productivity and uptake (productivity *vs.* change in exchangeable K r = -0.83, $P = 0.02$). At high initial concentrations of K and at higher rates of productivity, like inorganic N, K depletion is greater. This would indicate that while willow trees are taking up copious amounts of K when available such that the threshold concentration supporting high growth rates may be unsustainable. This is of concern for the maintenance of long term productivity, as K is also found to be correlated with greater leaf nutrition and stem growth (Chapter 3). At lower initial concentrations, the trees would seem to be effectively cycling K but this may be at suboptimal productivity.

4.5.5 Calcium and Sodium

Soil exchangeable Ca was found to be highly variable in many of the sites studied particularly when concentrations of exchangeable Ca were high. The demand by willow for Ca is reflected in the chemical composition of their biomass and seemingly in a decrease in soil Ca with establishment according to initial correlation analysis (Table 4.2). However, with the exception of LAK there was no change in soil Ca. Therefore, it is reasonable to conclude that some factor, other than the conversion to willow, caused the difference seen at LAK. As further evidence of this, there was no relationship between initial soil Ca or productivity and change in exchangeable Ca. The lack of change can be explained by the large quantities of total Ca at many of the sites (ELL, LAK, UOS, POR, BIR, GUE, PIC) included in this study. Calcium is also less prone to leaching than N and K following disturbance (Likens et al., 1994).

Due to interest in growing willow in marginal land unsuited to traditional agriculture (including sites affected by moderate salinity), the noted decrease in Na may prove to be beneficial. While only the UOS site had Ca:Na ratios <10, there was a decrease in Na in the upper 20 cm of soil across all sites where detectable quantities of Na were present (Figure 4.7). The increased water demand from the willow could be causing a decrease in the water table, resulting in the downward movement of soluble salts (Schofield, 1992). The ability of willow to decrease Na concentrations may make them useful for making use of saline soils not suited to agriculture or for phytoremediation (Hangs et al., 2011a).

4.6 Conclusions

4.6.1 Nutrient Cycling

Trees are generally efficient at recycling nutrients under natural conditions but in SRIC, this is disrupted by site disturbance, minimized understory development and removal of large quantities of biomass (instead of allowing the nutrients to be returned to the soil through decay of debris and death of trees). Site disturbance has been the dominant influence on soil nutrient status and C storage during the first three-year rotation. At this stage, the willow plantation has not yet reached its maximum growth potential. It is expected that productivity will rise to approximately 130% of the initial rotation in subsequent rotations and the roots system will continue to grow and mature (Volk et al., 2004). This will greatly increase rates of nutrient use and uptake, however, increasing productivity appears to mitigate effects of disturbance. Additionally, the majority of the willow in this study was not coppiced after the first year, as is typical. In a coppiced plantation increased biomass removal, disturbance and productivity may compound the effects of these factors.

After the first rotation, cycling of the various macronutrients is in different stages of recovery and efficiency. Increased demand for N has resulted in low levels of NO_3 thereby decreasing potential for leaching and denitrification. Likewise sites with greater growth had stabilized P concentrations; though P may be sensitive to routine disturbances such as coppicing. Thus an increasing litter layer may provide a sustained supply of N and P (Chen et al., 2000). Potassium has been shown to have a slow recovery from disturbance and as such biologically mediated processes have not been able to keep exchangeable K levels stable in optimal ranges early in establishment (Likens et al., 1994). In terms of plantation establishment, depletion of K and P was more heavily influenced by disturbance than direct effects of willow uptake as was N. The same biological cycling of nutrients which has kept P stable is much more difficult to achieve with K, likely due to its higher mobility.

4.6.2. Soil Carbon

There was a loss of soil C after the first three year rotation. Depending on management strategies and the previous land use this could be a temporary deficit although recovery times are

exacerbated in northern latitudes by low productivity (Laganière et al., 2010). The endpoint of short rotation intensive culture plantations in terms of soil C storage can be expected to be similar to other plantations given that soil disturbance is minimized. Reliance on chemical herbicides instead of between row tillage and harvesting in winter on frozen soils will contribute to reducing soil disturbance and maximizing the potential for soil C recovery. There is, however, little evidence as of yet that willow plantations have any potential to maintain or increase soil C when established on grasslands (native or pasture) or forests within the mineral soil. Plantations typically have depleted soil C when compared to these land uses (Guo and Gifford, 2002). However, the accumulation of C in the litter layer was not quantified in this study and may compensate for depleted mineral soil C (Paul et al., 2002; Pinno and Bélanger, 2008). SRIC may have an advantage over other plantations as frequent harvesting will leave behind some residue or slash.

Preface to Chapter 5

Regional land use change will have impacts on GHG balances. Willow production is no exception but because willow biomass is a potential C responsible energy feedstock it is doubly important to examine the effects of plantations on GHG balances. Indeed, greater productivity means greater atmospheric C sequestered and greater amounts of renewable energy produced. However, the full effects of this productivity in terms of GHG balances must be accounted. Chapter 3 and Chapter 4 examined the requirements for long term productivity of willow in Canada and, in particular, the potential need for amendments and changes to soil C. Chapter 5 continues the work begun in these chapters by examining the effect of willow on soil GHG efflux. Carbon dioxide emitted during the lifecycle of a plantation from fossil fuel consumption during site management, processing and transportation is readily calculable. This chapter fills a knowledge gap in the net emissions of GHGs from soils converted to willow production. It also addresses how varying rates of productivity affect these soil GHG balances.

5. EFFECTS OF WILLOW PLANTATION ESTABLISHMENT ON SOIL GREENHOUSE GAS DYNAMICS

5.1 Abstract

Willow (*Salix* spp.) is currently being researched as a source of bioenergy in Canada. These trees grow rapidly in high-density plantations and can be harvested as often as every three years. Short rotations, together with high energy output to input ratios, make willow promising as an energy feedstock. However, there is still much research to be done with willow in Canada to ensure net positive impacts on GHG balances throughout the full willow bioenergy life-cycle. It is well known that afforestation changes soil composition, structure and microclimate and therefore it is expected to alter GHG efflux as well. To test the effects of willow plantation establishment on soil GHG efflux, net fluxes of CO_2, N_2O and CH_4 were measured from the soil using vented chambers at two sites, one in and one near Saskatoon, SK. Measurements were taken from willow plantations, agricultural land and under mature trees. Soil respiration increased over time in the willow plantations as the trees grew larger (993-1092 kg C ha $^{-1}$ y^{-1} in 2007 to 1383-1586 kg C ha $^{-1}$ y^{-1} in 2009), shifting from an agricultural pattern to a mature tree pattern. Net CO_2 emissions were higher with higher plant productivity. Few and small fluxes of N_2O (0.01-0.28 kg N ha $^{-1}$ y^{-1}) and CH_4 (-0.34-0.03 kg C ha $^{-1}$ y^{-1}) were observed for any land use and as such cumulative fluxes were not different from each other ($P < 0.05$). Conditions in the soil were favorable for decreased emissions (cooler, drier, less inorganic N) but this did not translate into strong differences in GHG emissions from soils. Results indicate that willow plantations established on agricultural land do not adversely affect soil GHG efflux.

5.2 Introduction

5.2.1 Willow in a plantation setting

Traditional agricultural practices, tillage in particular, break up organic matter and make it more available for decomposition (Post and Kwon, 2000). Consequently, these practices have led to reductions in soil organic C levels (Lal, 2004). These same C-depleted soils, however, now represent a potentially very large sink for new C (Grogan and Matthews, 2002). For example,

conversion to no-till farming has pushed the balance back towards an increase in soil organic C (Lal, 2004). Short rotation willow plantations also are expected to transfer atmospheric C back into the soils (Heller et al., 2003), perhaps even exceeding the capacity of no-till agricultural systems. This is largely due to the growth of below ground biomass (roots), changes in litter quality (C:N ratios), shading and subsequent changes in microclimate, and lack of tillage in short rotation willow plantations.

Land-use change is usually accompanied by an initial decrease in soil C, regardless of the type and long-term net effect of the change (Guo and Gifford, 2002). Nevertheless, soil C stocks have been shown to increase when tree plantations are established on cropland, but decrease when native forest or pasture is converted to plantation (Guo and Gifford, 2002). Where there is a positive long-term change in soil C stocks, rates of increase can be expected to be greatest in the first years or decades after planting, although small gains are possible even after hundreds of years (Post and Kwon, 2000). The amount of C sequestered by short rotation intensive culture (SRIC) willow plantations is not only environmentally important, it is economically important as well. That is, practices that build soil organic-C pools represent a potential source of supplemental income to producers with trading of C offsets. In the case of short rotation willow plantations, this may help lessen the cost of willow-derived biomass fuels for consumers. Therefore, it becomes increasingly important to understand the potential of SRIC for sequestering C into long-term storage in the soils.

5.2.2 Carbon sequestration and greenhouse gas emissions

Sequestration of C into the soil is a balance between the addition and subsequent microbial decomposition of organic matter inputs (Batjes, 1998; Post and Kwon, 2000). Organic C, which begins as atmospheric CO_2, is captured by plants during photosynthesis and converted into organic forms. Plants then use a portion of this organic C for energy and in the process release CO_2 back into the atmosphere via plant respiration. When a plant or plant tissue dies, the organic C in the plant is added to the soil organic C pool where microbial decomposition occurs and CO_2 is released as a byproduct of microbial respiration. Thus, soil organic C stocks decrease

(and atmospheric CO_2 levels increase) if the rate of microbial decomposition exceeds the rate at which new plant-derived C is input into the soil ecosystem (Batjes, 1998).

Microbial activity, and therefore microbial respiration, is governed by soil conditions. Hot, moist conditions are optimal for microbial activity and so GHG efflux increases under these conditions. As well, an abundance of nutrients and soil C increases microbial respiration and denitrification (Wrage et al., 2001). Traditional agriculture increases N availability through fertilization and breaks up soil aggregates such that soil C is more accessible for microbial activity. However, these same nutrient rich conditions promote rapid tree growth, thereby increasing the amount of atmospheric C incorporated into new biomass and its subsequent sequestration in the soil. Moreover, because trees shade and dry the soil and reduce the need for both tillage and fertilizer, it is to be expected that planting trees on agricultural land will reduce GHG efflux and increase C sequestration.

Whereas general trends in soil C and GHG efflux in plantations have been described in the literature (Laganière et al., 2010), tree plantations are relatively new to the Canadian prairies and so direct measurements of soil GHG efflux in plantations are rare for this region. In addition, SRIC introduces a somewhat different set of circumstances; e.g., willow grown in SRIC is planted at very high densities (>15,000 stems ha^{-1}) and the rotations are far shorter (3 years) than in other continental temperate tree plantations (10-12 years for short rotation forestry (e.g. *Populus*) or >30 years for traditional forestry (e.g. *Pinus*) (Weih, 2004). Thus, it is not unrealistic to think that the trends observed in non-SRIC plantations may differ from those seen in a SRIC plantation. In short, C sequestration in plantations is controlled by a complex system of interactions between trees, soil, climate and other environmental factors. Thus, there is a need to expand knowledge of how plantations influence GHG emissions to include willow grown in SRIC on the Canadian prairies—especially in light of the potential for growth in the industry.

Nitrous oxide (N_2O), although much lower in atmospheric concentration than CO_2, is an extremely potent GHG—with a global warming potential (100-yr timeframe) that is 296 times greater than that of CO_2 [Intergovernmental Panel on Climate Change (IPCC), 2007]. Moreover, approximately 0.2–1% of all synthetic N fertilizer used in the Northern Great Plains (which includes the Canadian prairies) is released to the atmosphere as N_2O (Lemke et al., 2010).

Nitrous oxide is produced primarily during denitrification, although other pathways (e.g., nitrification) can be important under certain circumstances (Bremner and Blackmer, 1981). Under oxygen limited conditions, some bacteria can use nitrate (NO_3^-) as an electron receptor as part of their metabolism. The NO_3^- is progressively reduced to nitric oxide (NO), N_2O, and ultimately N_2—all of which can be released to the atmosphere. Thus the amount of N_2O produced is largely regulated by the O_2 status of the soil, which is both temporally and spatially highly variable due in large part to the effects of soil moisture (Yates et al., 2006). In a SRIC willow plantation it can be expected that the soil will be drier and cooler, with less available N; thus, N_2O emissions should be lower in the plantation when compared to a traditional cropping system.

Methane also is an important GHG, with a global warming potential (100-yr timeframe) that is 23 times greater than that of CO_2 [Intergovernmental Panel on Climate Change (IPCC), 2007], and is produced under highly anaerobic conditions. Thus, with the exception of wetland soils and soils that become temporarily saturated, most agricultural and forest soils are sinks for CH_4. Indeed, CH_4 oxidation is typically greatest in forested soils (Le Mer and Roger, 2001), which suggests that it may also be high in plantation soils.

5.2.3 Greenhouse gas budgets

Greenhouse gas budgets for willow bioenergy plantations are available from the literature (Grogan and Matthews, 2002; Heller et al., 2003); however, they tend to lack the data needed to evaluate changes in soil C stocks and GHG emissions from soil. Moreover, because SRIC plantations are relatively new to Canada, there is a large knowledge gap for this region and a need to make direct measurements of GHG efflux and soil organic C stocks in SRIC willow and matched reference sites.

This chapter addresses the gap in the knowledge of how SRIC willow impacts GHG emissions and, ultimately, C-sequestration. This research tests the hypothesis that willow plantations alter GHG balances for the better (compared with traditional agriculture) by reducing CH_4, N_2O, and CO_2 emissions and increasing soil C stocks due to lower soil temperatures and moisture content.

5.3 Materials and Methods

Life-cycle analysis of SRIC willow plantations requires a knowledge of the changes in total soil C after establishment, as well as changes to GHG efflux regimes resulting from alterations to land use patterns. Soil C measurements were conducted in conjunction with the work described in Chapters 3 and 4. Greenhouse gas (CO_2, N_2O or CH_4) fluxes were measured at the University of Saskatchewan (UOS) willow plantation—located in Saskatoon, SK, Canada (52°07'N 106°36'W; planted 2006)—from spring 2007 through autumn 2009 and the Lakeshore Tree Nursery (LAK)—located near Saskatoon, (52°00'N 106°45'W; planted 2005)—from spring 2007 to autumn 2007. The reference sites at UOS included an agricultural field adjacent to the willow plantation, and is representative of site at the time the plantation was established as well as a pure stand of willow planted >15 years before. At LAK, the reference sites included an agricultural field and a naturally occurring mixed species forest. Willow was planted in a triple row design with 60 cm between trees and 200 cm between rows.

Plant available nitrate (NO_3^-) and ammonium (NH_4^+) and total soil C were measured at both the plantation and reference sites in the third year from establishment at UOS and LAK as well as the seven other sites described in greater detail in Chapters 3-4 (Table 5.1). While GHG measurements were only feasible at UOS and LAK, the larger dataset provides a better test of willow's ability to remove substrate for denitrification. Soil from 0-20 cm and 20-40 cm (n = 3 for each plot and depth) were collected in early August and returned to the laboratory where they were air dried and sieved to pass a 2-mm mesh screen. Nitrate and NH_4^+ were extracted using a 2.0 *M* KCl solution and analyzed colorimetrically on a Technicon Auto-Analyzer (Technicon Instruments Corporation, Tarrytown, NY). Total soil C was measured on a LECO C Analyzer at 1100°C (LECO Corporation, St. Joseph, MI).

Table 5.1: Site locations, productivity and soil inorganic N and total soil C for plantations and reference sites for nine *S. purpurea* 'Hotel' plantations (ELL, SMO, LAK, UOS, POR, BIR, SSM, GUE, PIC).

ID	Geographic Coordinates	Harvested Biomass	NO_3^-		NH_4^+		Total Soil Carbon	
			Willow	Ref.	Willow	Ref.	Willow	Ref.
		Mg ha^{-1}	μg N g^{-1}				mg g^{-1}	
Ellerslie Research Station, AB (ELL)	53°25'N 113°31'W	N/A	4.20	2.79	8.97	8.37	68.8	70.7
Smoky Lake, AB (SMO)	54°07'N 112°24'W	5.4 (0.1)	1.82	0.14	4.28	2.48	21.2	22.0
Lakeshore Tree Nursery, SK (LAK)	52°00'N 106°45'W	18.4 (1.2)	0.84	6.84	0.74	1.58	35.0	35.3
University of Saskatchewan, Saskatoon, SK (UOS)	52°07'N 106°36'W	5.3 (0.8)	0.22	1.58	13.86	16.05	24.2	28.0
Portage la Prairie, MB (POR)	49°57'N 98°10'W	22.8 (3.2)	0.58	13.85	6.30	8.14	38.4	37.0
Bird's Hill, MB (BIR)	50°00'N 97°00'W	N/A§	1.15	4.13	7.31	7.04	42.1	48.1
Sault Ste Marie, ON (SSM)	46°32'N 84°24'W	6.1 (0.3)	1.05	0.09	10.39	11.47	22.7	27.2
Guelph, ON (GUE)	43°33'N 80°13'W	9.5 (2.5)	1.61	1.87	8.33	6.87	24.3	29.0
Pickering, ON (PIC)	44°00'N 79°01'W	14.0 (2.1)	1.14	0.86	7.81	14.54	22.2	21.5

Greenhouse gas efflux was measured throughout the growing season, and continued until freeze-up (May–October 2007, 2008, and 2009 at UOS and May-October 2007 at LAK). Measurements were taken at least once per week throughout the growing season, with the sampling schedule adjusted to include periods when gas emissions were most probable; e.g., after rainfall. Soil temperature and moisture was recorded each time GHG effluxes were measured from immediately surrounding each sampling point in 2007. Soil moisture to 12 cm was measured using a Field Scout time domain reflectometry 300 probe (Spectrum Technologies, Plainfield, Illinois). Soil Temperature at 10 cm was measured via *in situ* thermocouples. Daily total precipitation amounts were gathered from Environment Canada weather data from Saskatoon, SK. Ambient air temperatures were measured using thermocouples.

Gas efflux was sampled using non-steady state, vented chambers (surface area = 201 cm^2, internal volume = 2252 cm^3) (Yates et al., 2006) over the course of three growing seasons at UOS starting in the second year from establishment (2007) and one growing season at LAK in the third year after establishment (2007). Five collars were installed in each of the following: the 'Hotel' clone of *S. purpurea*, an alternate *Salix* clone [either 'Acute' (LAK) or 'Charlie' (UOS)], agricultural land, and under mature trees [pure mature stand (UOS) or natural forest (LAK)]. On each sampling date, a chamber was sealed to the collar and gas samples were collected at 8 min intervals for a total of 24 min. Gas samples were collected from the enclosed headspace using a disposable, 25 cc syringe equipped with a 25 gauge, 5/8 inch needle; were immediately injected into pre-evacuated (*ca*. 0.005 atm), 12 cc Exetainer™ vials; and analyzed using gas chromatography (Farrell et al., 2002). Gas sampling commenced as soon after snow melt as was feasible and continued into the fall (i.e., until ground freeze-up). Sampling intensity was greatest during the summer months so as to capture the greatest amount of activity and variability. Greenhouse gas fluxes were determined by fitting a linear least-squares regression model to the gas concentration *vs*. time data (Yates et al., 2006) and then calculating the flux (F_g) as described by Rochette and Bertrand (2008):

$$F_g = dG/dt \times V/A \times M_{m,g}/V_m \times (1-e_p/P) \quad [5.1]$$

where dG/dt (mol mol^{-1} s^{-1}) is the change in gas concentration within the chamber during deployment and is calculated from the slope of the linear regression; V (m^3) is the volume of the chamber; A (m^2) is the area under the chamber; $M_{m,g}$ (g mol^{-1}) is the molecular mass of the gas under consideration (i.e., CO_2, N_2O or CH_4); V_m (m^3 mol^{-1}) is the molar volume of the gas at the temperature and atmospheric pressure recorded at the time of deployment (i.e., at t = 0); e_p (kPa) is the partial pressure of water vapor in the chamber headspace; and P (kPa) is the ambient (barometric) pressure.

Using linear regression in the chamber method is known to frequently underestimate GHG fluxes as increasing concentrations of gases within the chamber may decrease the diffusion gradient such that continued emission from the soil is suppressed (Rochette and Bertrand, 2008).

Non-linear models may be applied in such cases to provide a better estimate. However, non-linear models, while providing a better fit, are not always appropriate and are more sensitive to error (Rochette and Bertrand, 2008). This can result in a mix of models being applied to the same dataset. While this may produce a cumulative value closer to the true value, fluxes calculated by non-linear methods are substantially higher than those calculated by linear models. Therefore, in comparative studies, such as this, what is being compared may actually be an artifact from differences in the frequency of non-linear models rather than true differences of the fluxes themselves. Thus, only linear models were used in calculating fluxes to maintain comparability at the expense of underestimation.

Ambient air samples were included as reference samples in each analytical run to check the 'within run' precision, calculate the minimum detectable concentration difference (MDCD), and correct for detector drift. The MDCD was calculated for each sampling day by (i) analyzing matched pairs of the reference gas samples at regular intervals during each analytical run; (ii) calculating the average difference between sample pairs ($\mu_{pair\ diff}$) as well as the standard deviation ($\sigma_{pair\ diff}$); and (iii) calculating the MDCD using Eqn. [2] : (Yates et al., 2006)

$$MDCD = \mu_{pair\ diff} + (2\sigma_{pair\ diff}) \quad [5.2]$$

As is common with datasets that include large numbers of "non-detects", distinguishing non-significant fluxes (e.g. below the MDCD) from natural variability as well as sampling error proved difficult. Although reporting the calculated value of any single non-significant flux as a true value may be erroneous it is equally erroneous to "left censor" by setting that value to zero or any arbitrary fraction of the MDCD within a larger dataset (Helsel, 2006), as is common practice in soil GHG efflux studies (Yates et al., 2006). Therefore non-significant fluxes were included within the dataset to minimize left censoring. Due to the large sample size in this study, statistical analyses performed on cumulative fluxes are still valid assuming that noise from including non-significant fluxes is normally distributed.

Graphic interpretation allowed for characterization of temporal patterns. The mean and standard errors for each site and land use were determined from the individual estimates.

Cumulative (yearly) GHG emissions were calculated for each plot (replicate) by linearly interpolating data points and integrating the underlying area (Bronson and Mosier, 1993; Lemke et al., 1999). Significant differences in yearly cumulative GHG efflux between land-uses were evaluated using the Student's t-test.

5.4 Results and Discussion

5.4.1 Microclimate

Climate data for the UOS site during the study period (May 2007–October 2009) are presented in Figure 5.1. In general, whereas growing season temperatures were slightly warmer (1–3°C) than the long-term (30-yr) average for all four years, growing season precipitation varied considerably from year to year (Table 5.2). Notably, the 2008 growing season was characterized by a larger than normal moisture deficit (-26%).

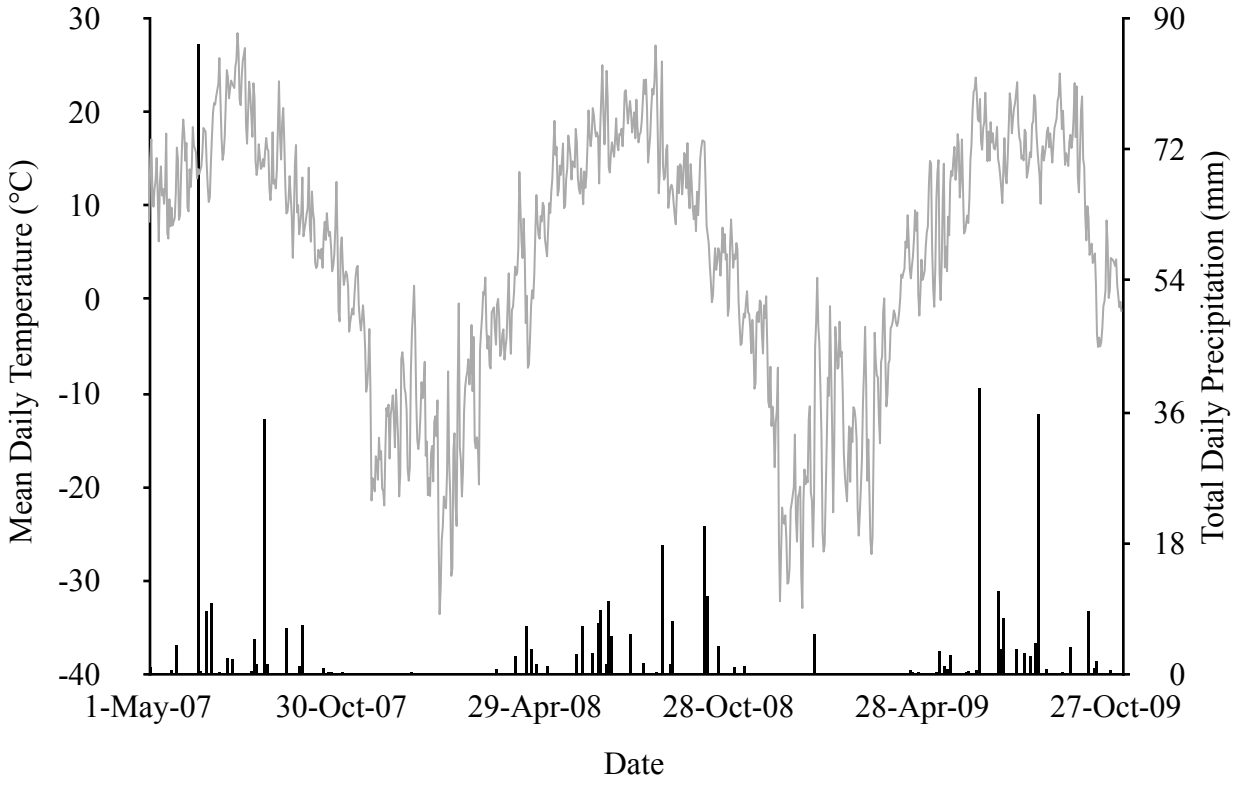

Figure 5.1: Mean daily temperature and precipitation data for the University of Saskatchewan (UOS) willow plantation during the period from May 2007 through October 2010. Bars indicate total daily precipitation. The line indicates mean daily temperature.

Table 5.2: Summary of University of Saskatchewan (UOS) willow plantation climate for years of soil GHG efflux study.

Year	Growing season† air temperature (°C)	ΔT‡ (°C)	Growing season† precipitation (mm)	ΔP§ (mm)
Long-term Average¶	13.3	—	227.4	—
2007	16.1	+2.8	297.4	+70.0
2008	15.3	+2.0	168.8	-58.6
2009	15.1	+1.8	225.7	-1.8

†Average daily temperature or cumulative precipitation during the period from May 1st – September 30th.
‡Difference in the average daily temperature relative to the long-term average.
§Difference in the cumulative growing season precipitation relative to the long-term average.
¶Calculated from the Canadian climate normals (1971–2000) (Environment Canada, 2011).

The presence of trees in a landscape is expected to decrease both the soil water content (by increasing evapotranspiration) and soil temperature (by shading) (Binkley, 1995), which in turn should decrease the production and soil-to-atmosphere transfer of CO_2, N_2O, and CH_4 (Le Mer and Roger, 2001; Yates et al., 2006; Laganière et al., 2010). Planting willow had a cooling and drying effect on the soils versus agriculture in the second (UOS) and third (LAK) years of growth (Figure 5.2). This effect was much more pronounced in the LAK plantation where willow was one year older and much more productive. Soil under mature trees was cooler and drier yet.

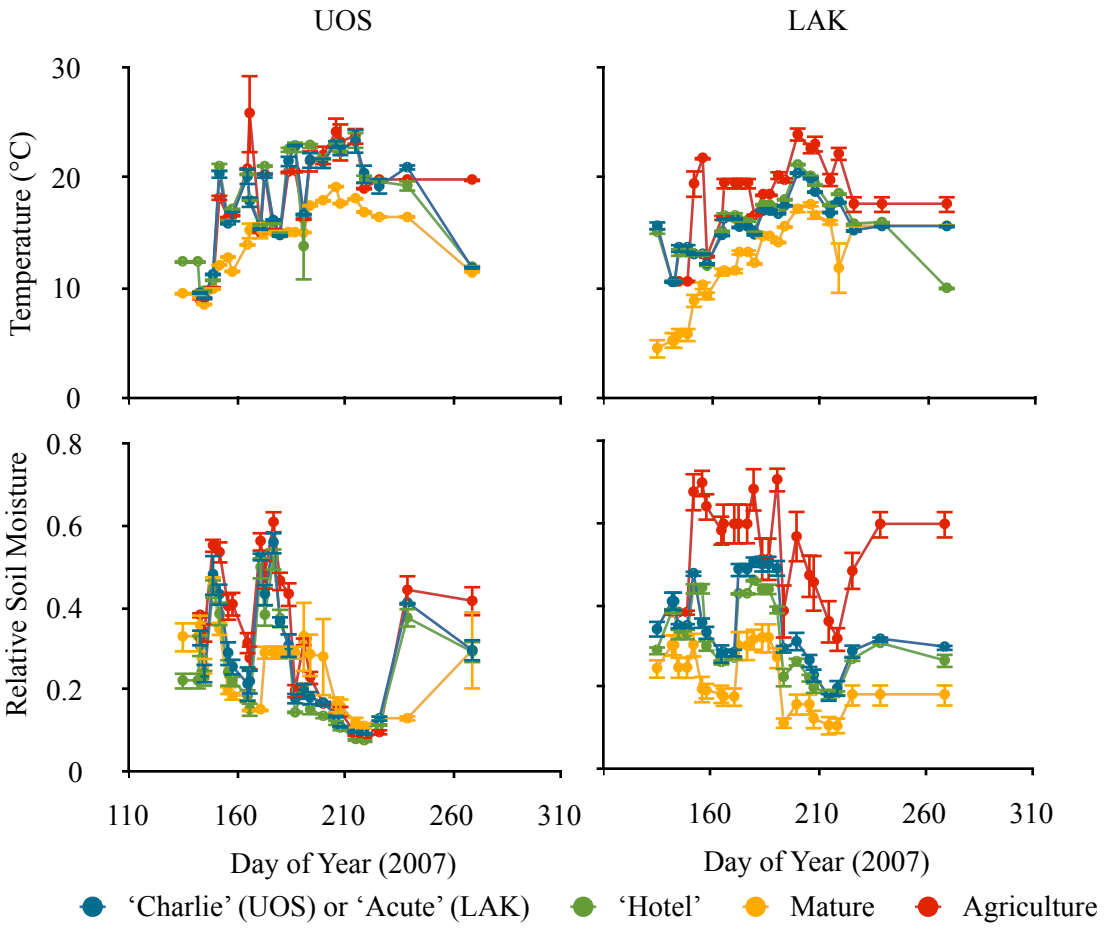

Figure 5.2: Relative soil moisture and soil temperature under annual cropping (Agriculture), two clones of willow in plantation ('Charlie' or 'Acute' and 'Hotel') and mature stand (Mature) for 2007 at UOS and LAK sites.

5.4.2 Nitrous oxide

As is typical, very few detectable fluxes of N_2O were recorded for any land use (Yates et al., 2006). Most fluxes that were detected were very small (< 5 ng N_2O-N m^{-2} s^{-1}) as would be expected for background efflux (Figures 5.3-5.6). Previous studies have linked nitrous oxide emission with wet soils having water filled pore space in the range of 60-80% (Yates et al., 2006). This would suggest that emissions would most likely occur after precipitation events. However, only one emission event seems to be directly tied to precipitation with the most pronounced effect in the agriculture and 'Charlie' plots at UOS (Figure 5.3). Similarly, larger

N_2O fluxes were recorded in the agricultural land use at LAK in 2007 (Figure 5.6) and UOS in 2008 (Figure 5.4) with neither of these fluxes being apparent in other land uses. In 2009, no larger emission events occurred in any land use (Figure 5.5).

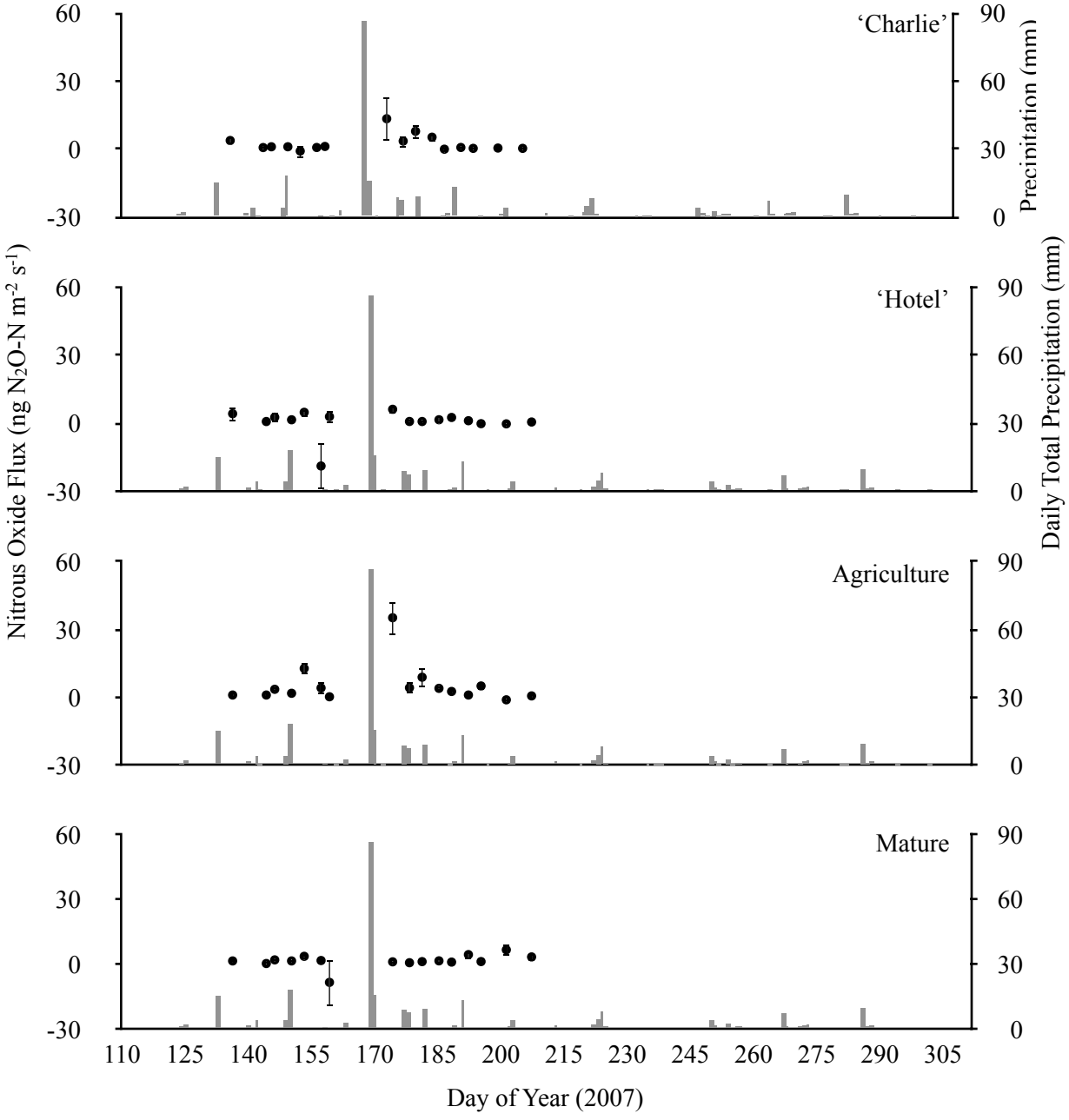

Figure 5.3: Nitrous oxide fluxes from soil (ng N_2O-N m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) and daily precipitation totals (mm) (bars) for UOS in 2007 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

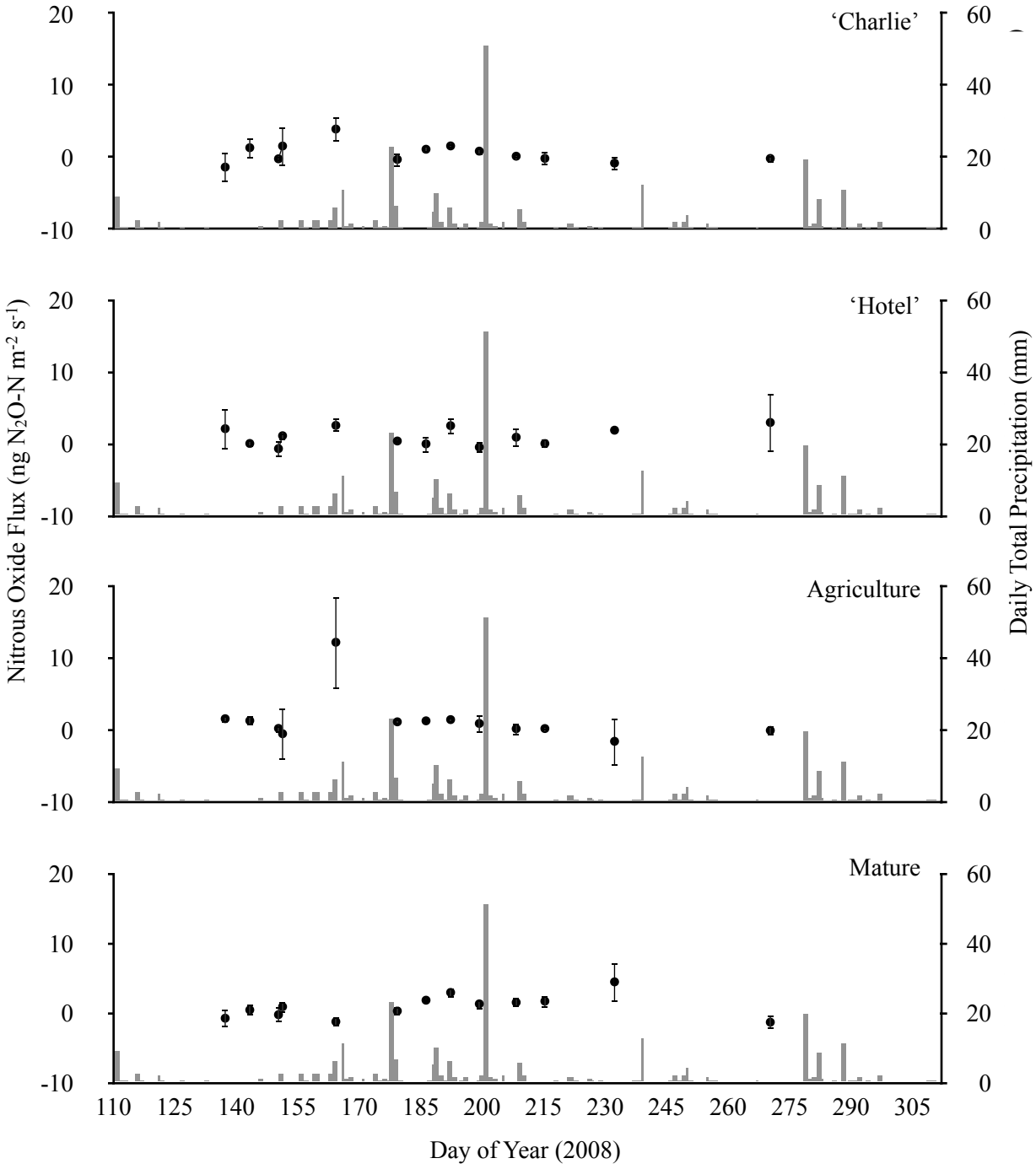

Figure 5.4: Nitrous oxide fluxes from soil (ng N_2O-N m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error,) and daily precipitation totals (mm) (bars) for UOS in 2008 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

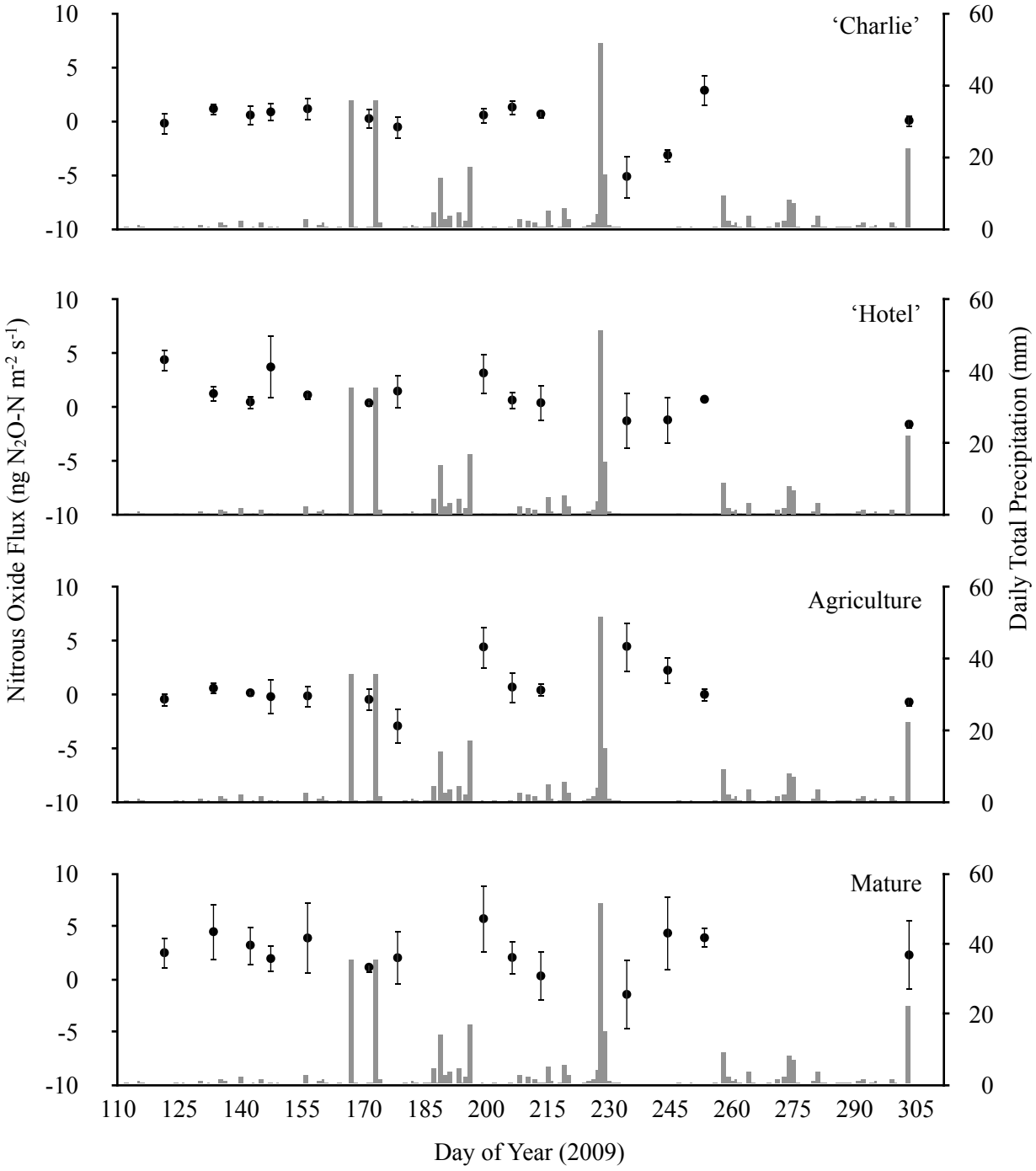

Figure 5.5: Nitrous oxide fluxes from soil (ng N_2O-N m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error,) and daily precipitation totals (mm) (bars) for UOS in 2009 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

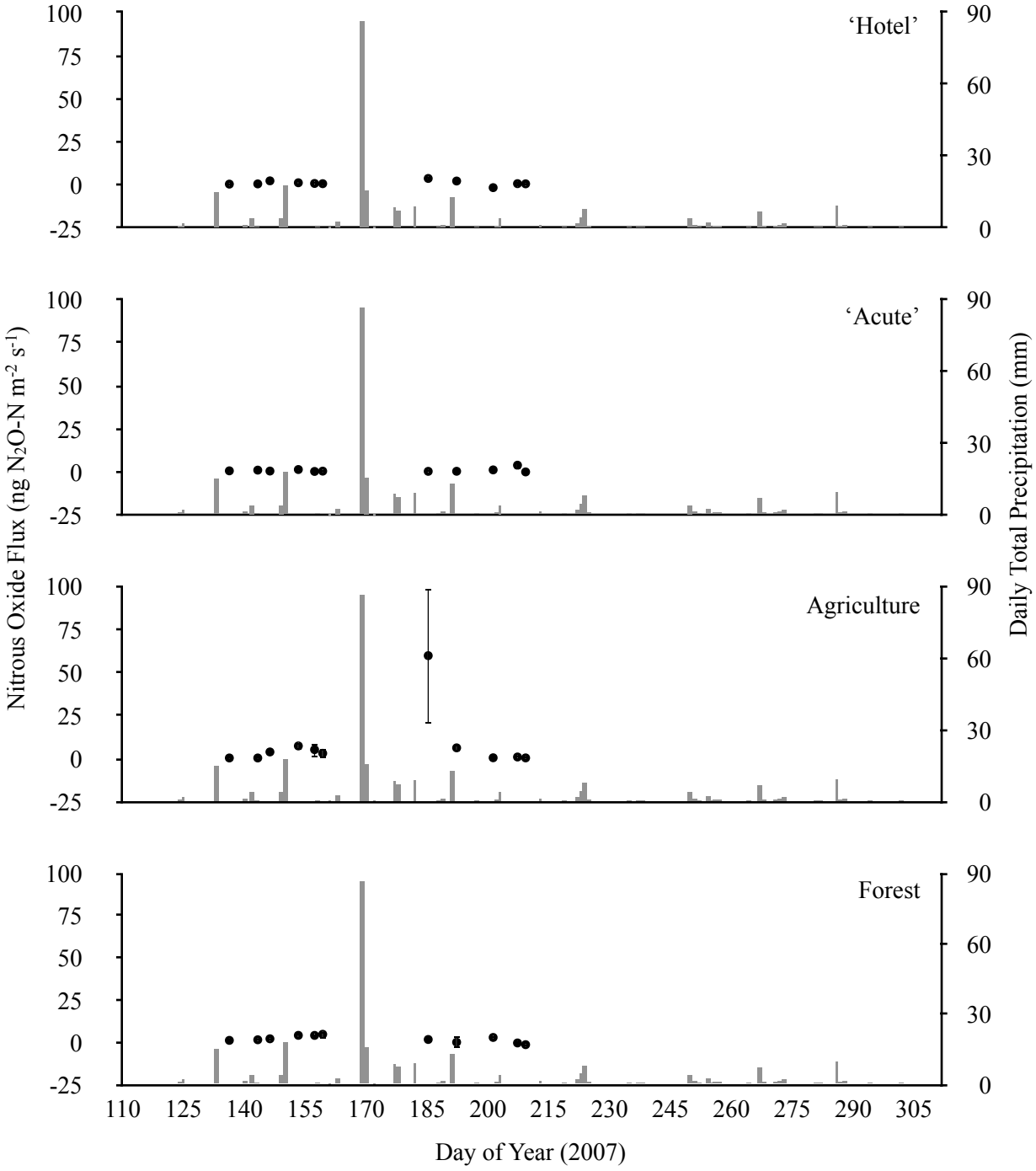

Figure 5.6: Nitrous oxide fluxes from soil (ng N_2O-N m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error,) and daily precipitation totals (mm) (bars) for LAK in 2007 for two clones of willow ('Acute' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

Fewer N_2O efflux events were observed in plantation willow as compared to the agricultural soil and mature willow over three years but again very few substantial events were recorded for any land use (Figure 5.7). As such, there was no significant difference between the land uses in the number of events or cumulative N_2O emissions. Although no difference was detected in cumulative N_2O emissions (Table 5.3), in theory the cooler drier plantation soils would be less prone to N_2O release. Further reducing the risk of N_2O efflux is the depletion of the nitrate in the soil with increasing productivity as seen in Figure 5.8.

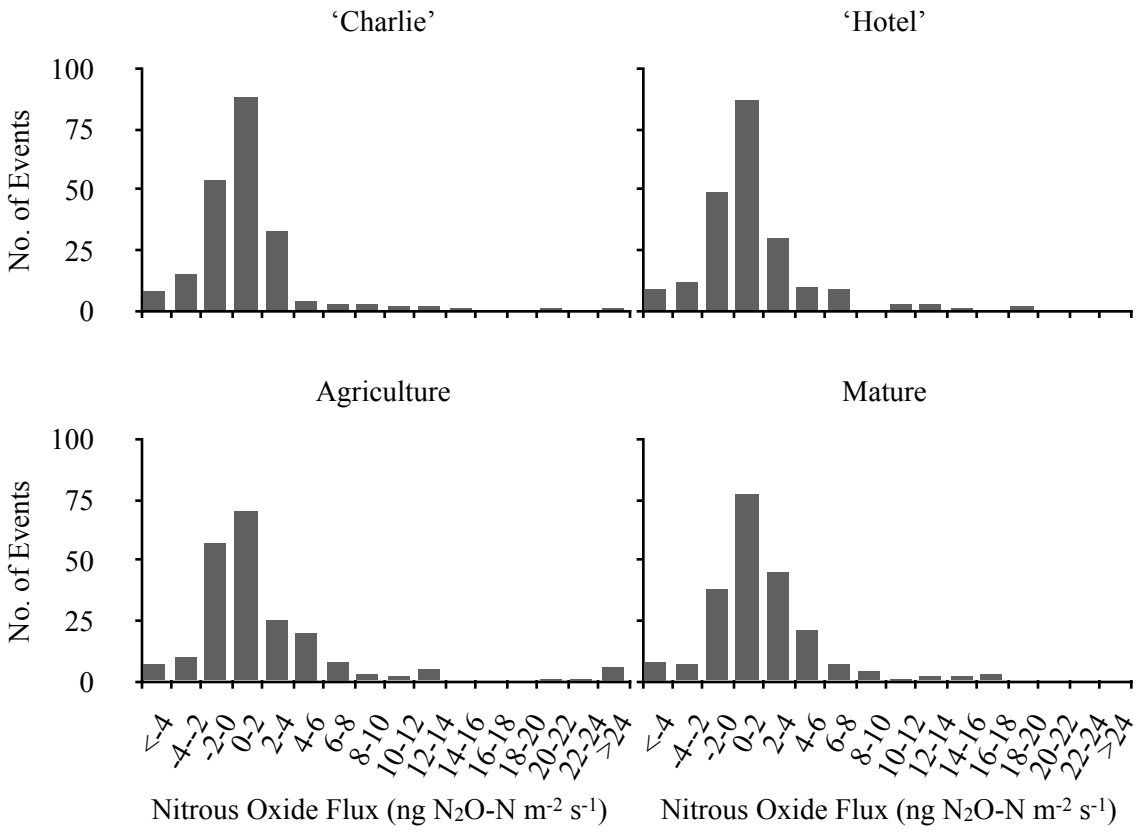

Figure 5.7: Measured N_2O flux from soil (ng N_2O-N m^{-2} s^{-1}) event frequencies by treatment at UOS in 2007-2009 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

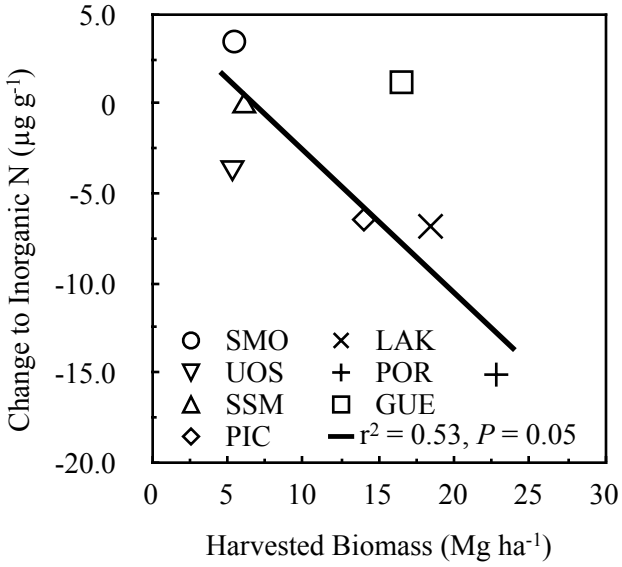

Figure 5.8: Harvested biomass (Mg ha^{-1}) vs. change in inorganic N (µg g^{-1}) after the first three year rotation at seven *S. purpurea* 'Hotel' plantations (SMO, LAK, UOS, POR, SSM, GUE, PIC).

Table 5.3: Mean cumulative GHG emission/sequestration from/into soil for UOS and LAK sites for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agric.) and mature trees (Mature) described by individual gas and total 100-yr global warming potential CO_2-C equivalent .

	N_2O		CH_4		CO_2	Total GHG
	N_2O-N (SE)	CO_2eq-C (SE)	CH_4-C (SE)	CO_2eq-C (SE)	CO_2-C (SE)	CO_2eq-C (SE)
	kg ha^{-1}					
2007 - UOS						
Charlie	0.12 (0.07)a†	36 (21)a	-0.08 (0.05)a	-2 (1)a	993 (129)a	1,027 (145)a
Hotel	0.05 (0.02)a	15 (6)a	-0.16 (0.13)a	-4 (3)a	1,092 (93)ab	1,103 (91)a
Agric.	0.28 (0.05)a	82 (16)a	-0.05 (0.04)a	-1 (1)a	1,473 (147)b	1,554 (154)b
Mature	0.03 (0.06)a	9 (19)a	0.03 (0.02)a	1 (1)a	1,345 (84)ab	1,354 (78)ab
2008 - UOS						
Charlie	0.04 (0.03)a	11 (10)a	-0.14 (0.14)a	-3 (3)a	1,167 (87)a	1,175 (93)a
Hotel	0.10 (0.04)a	28 (13)a	-0.18 (0.07)a	-4 (2)a	1,037 (163)a	1,061 (171)a
Agric.	0.09 (0.08)a	27 (23)a	-0.34 (0.06)a	-8 (1)a	1,518 (119)b	1,538 (131)a
Mature	0.09 (0.05)a	25 (15)a	-0.12 (0.18)a	-3 (4)a	1,371 (121)ab	1,394 (107)a
2009 - UOS						
Charlie	0.01 (0.05)a	4 (15)a	-0.06 (0.02)a	-1 (1)a	1,586 (142)a	1,588 (129)a
Hotel	0.07 (0.04)a	20 (11)a	-0.11 (0.01)a	-3 (0)a	1,383 (242)a	1,400 (239)a
Agric.	0.06 (0.06)a	17 (17)a	-0.12 (0.04)a	-3 (1)a	1,453 (96)a	1,467 (109)a
Mature	0.26 (0.16)a	77 (46)a	-0.04 (0.03)a	-1 (1)a	1,520 (123)a	1,596 (97)a
2007 - LAK						
Agric.	0.64 (0.35)a	191 (105)a	-0.11 (0.08)a	-2 (2)a	1,026 (138)a	1,215 (196)a
Forest	0.09 (0.04)a	27 (11)a	-0.24 (0.05)b	-5 (1)b	1,634 (223)b	1,656 (227)a
Hotel	0.06 (0.03)a	19 (9)a	-0.16 (0.11)a	-4 (3)a	1,168 (80)ab	1,183 (86)a
Acute	0.03 (0.00)a	9 (1)a	-0.03 (0.07)a	-1 (2)a	1,129 (106)ab	1,138 (106)a

† Within column, site and year, means followed by the same letter are not significantly different ($P < 0.05$)

5.4.3 Methane

Fluxes of CH_4 were typically small (-0.05-0.05 μg CH_4-C m^{-2} s^{-1}) and negative (Figures 5.9-5.12). There was little oxidation of CH_4 at any time of year in the mature willow stand at UOS with some emission events also being detected but this may have been due to ponding of the water on the soil surface rather than a direct land use effect. Saturated soils decreased the net amount of CH_4 consumed by methanotrophs within the mature stand soil. At the LAK site, the

forested plots exhibited a more typical negative efflux of CH_4 greater than the other land uses ($P < 0.05$). The willow plantations and the agricultural field all experienced net negative fluxes for CH_4 (Table 5.3). Cumulative CH_4 efflux was not different at the end of the growing season with the exception of the LAK forest, however there were subtle differences in patterns exhibited by each land use. At UOS, for the willow plantations, the decrease in negative fluxes coincided with the degree of cover given by the particular clone. The 'Hotel' clone being much smaller at this site than the 'Charlie' clone did not provide much shade until later in the growing season. This led to greater CH_4 oxidation in the 'Hotel' clone early in the year, most evident in 2007 (Figure 5.13). Methane oxidation in the agricultural field was greatest after harvest (Figure 5.13). Harvesting may allow the remaining stubble to act as a pathway for CH_4 infiltration into the soil much in the same way that rice plants are known to be a pathway for CH_4 release (Le Mer and Roger, 2001). However, this trend as seen in 2008 could also be the effect of an exceptionally hot and dry fall to which the bare agricultural soil responded most (Table 5.2).

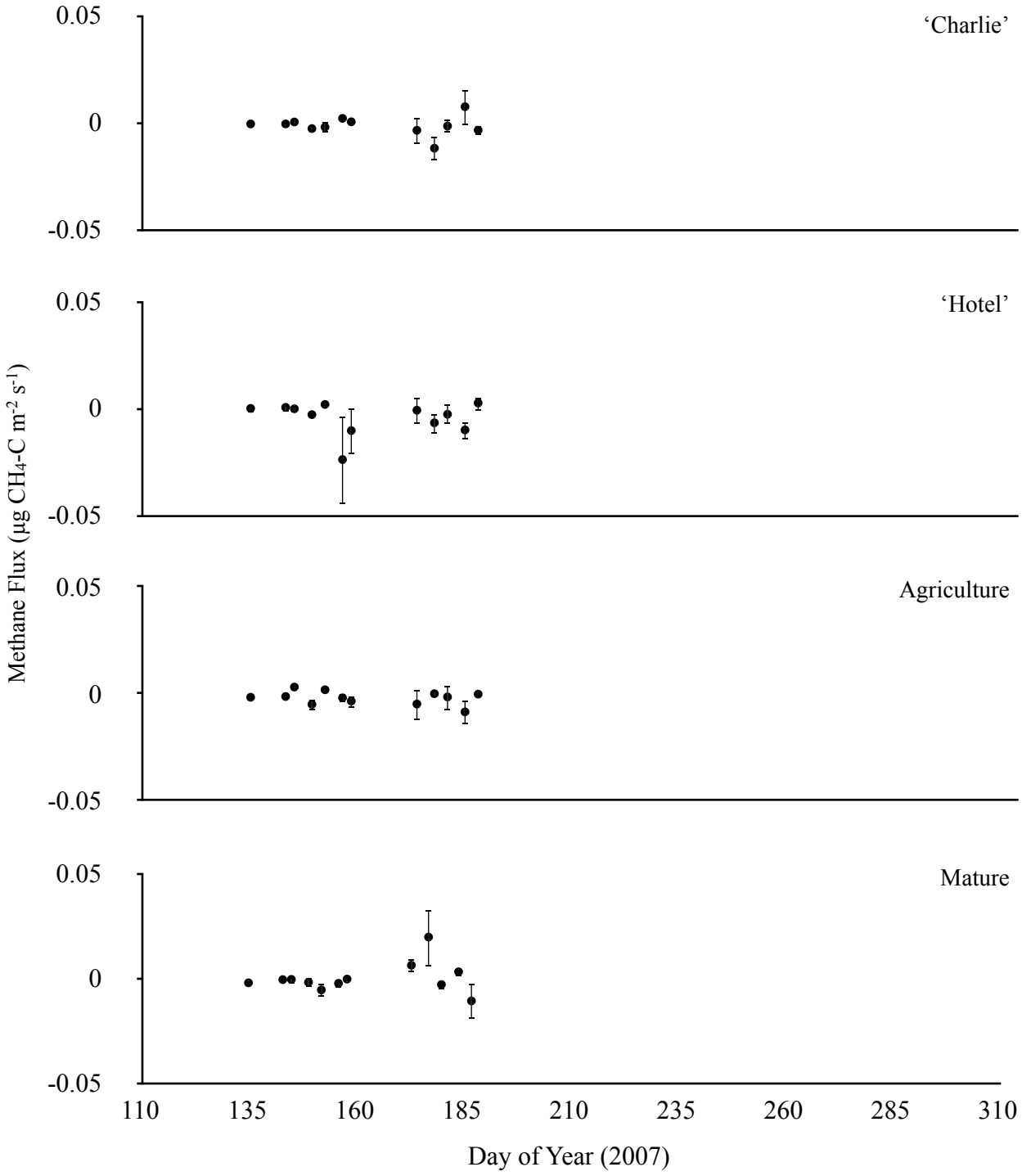

Figure 5.9: Methane fluxes from soil (µg CH_4-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for UOS in 2007 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

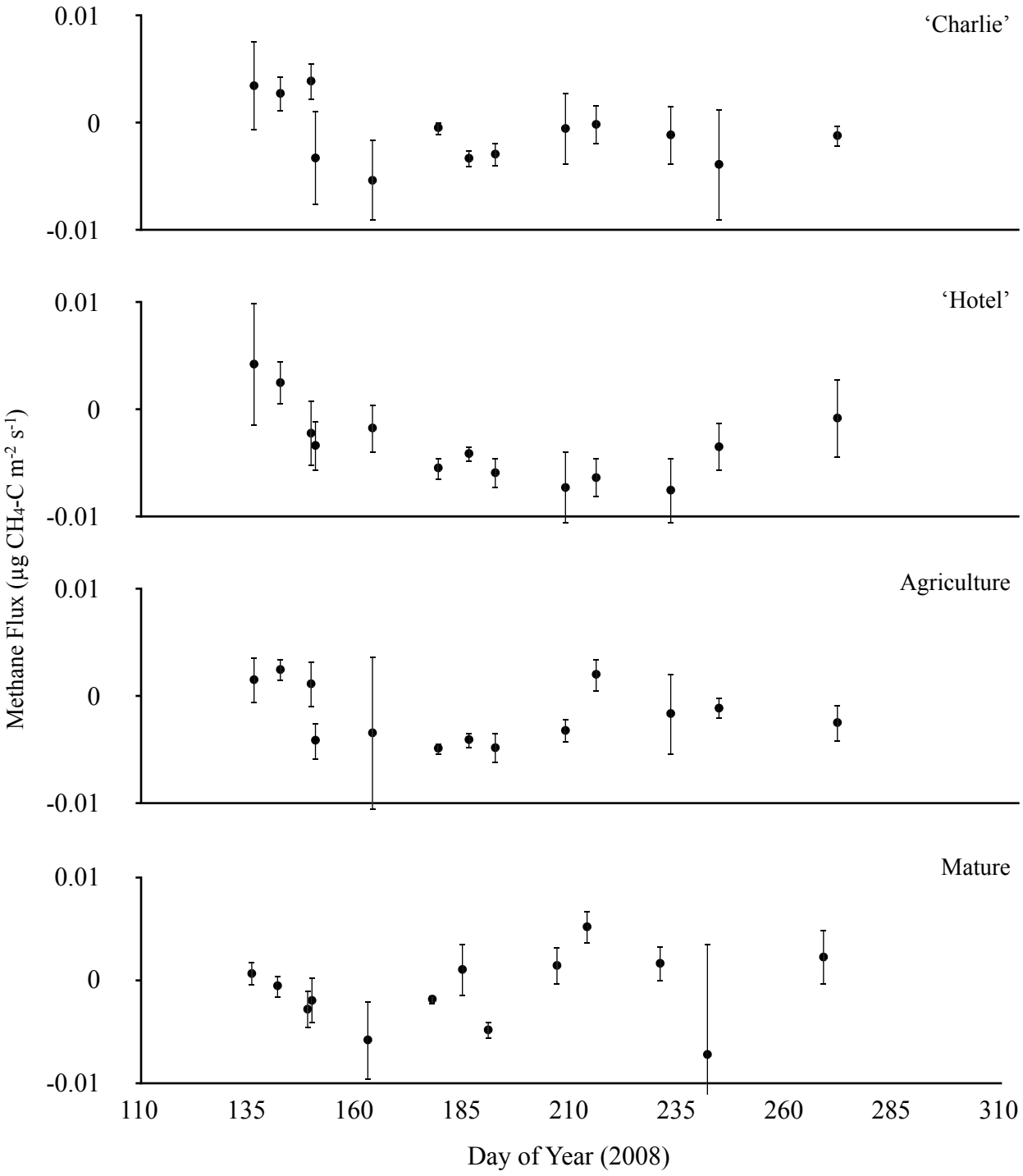

Figure 5.10: Methane fluxes from soil (µg CH_4-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for UOS in 2008 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

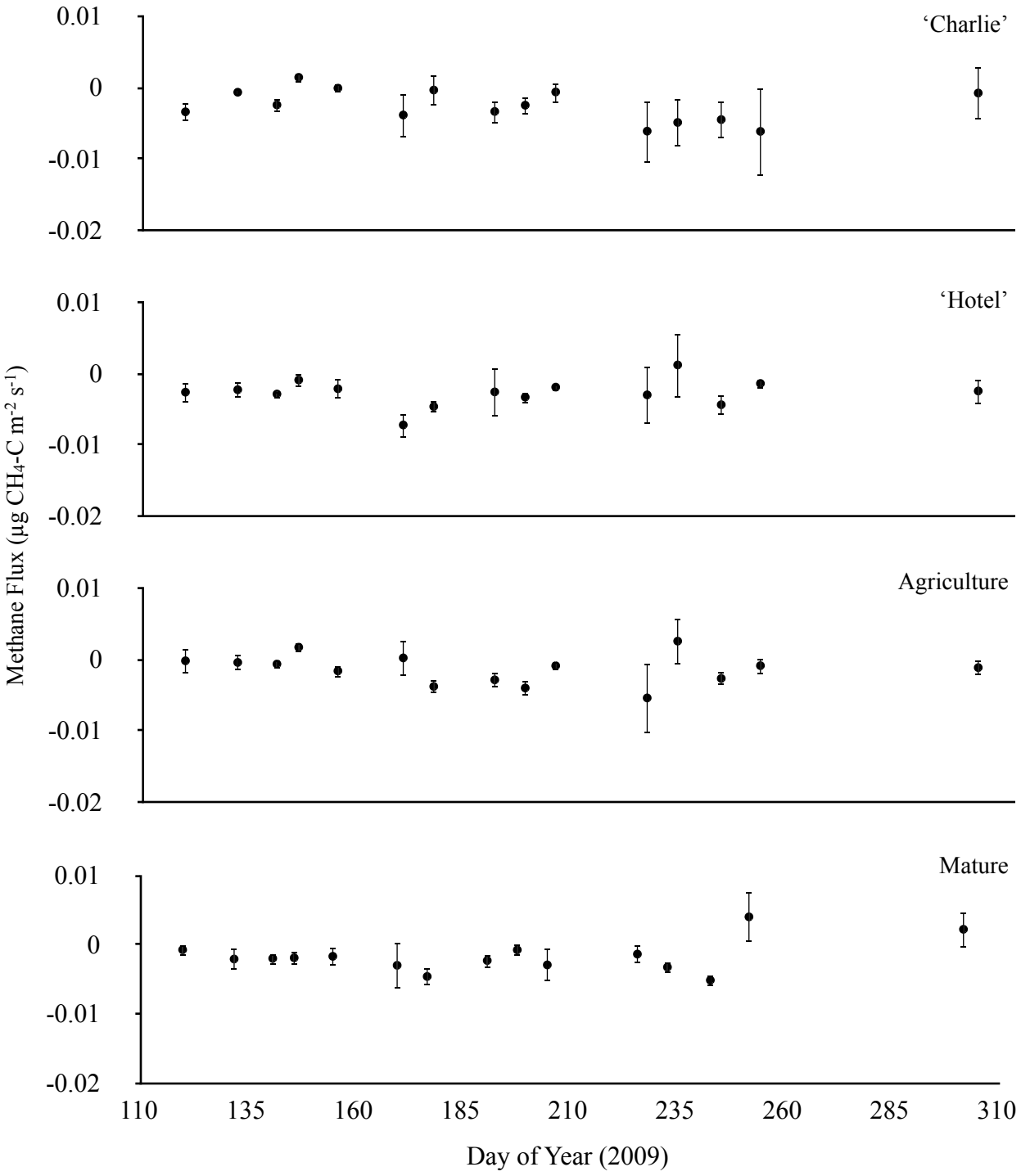

Figure 5.11: Methane fluxes from soil (µg CH_4-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for UOS in 2009 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

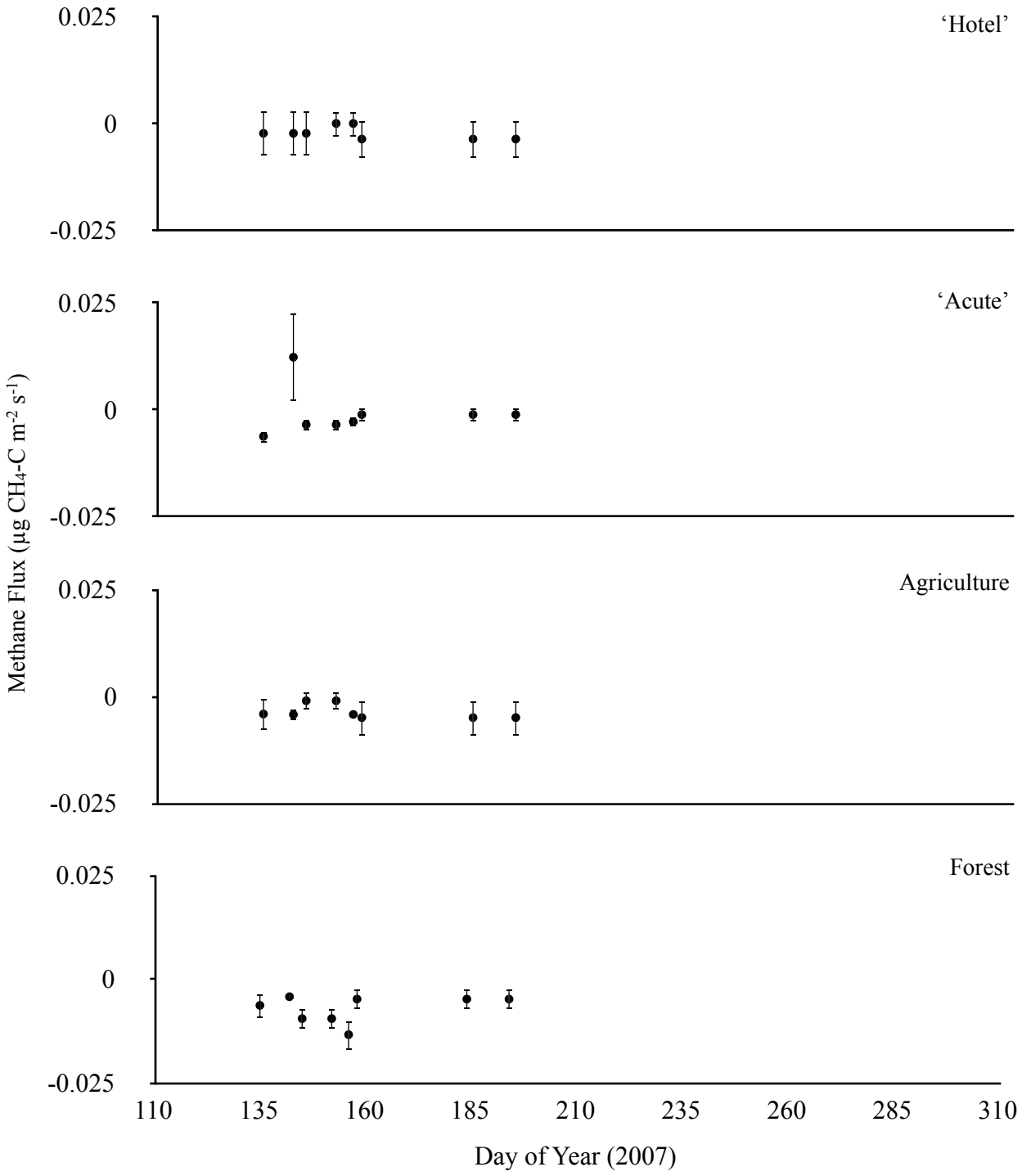

Figure 5.12: Methane fluxes from soil (µg CH_4-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for LAK in 2007 for two clones of willow ('Acute' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

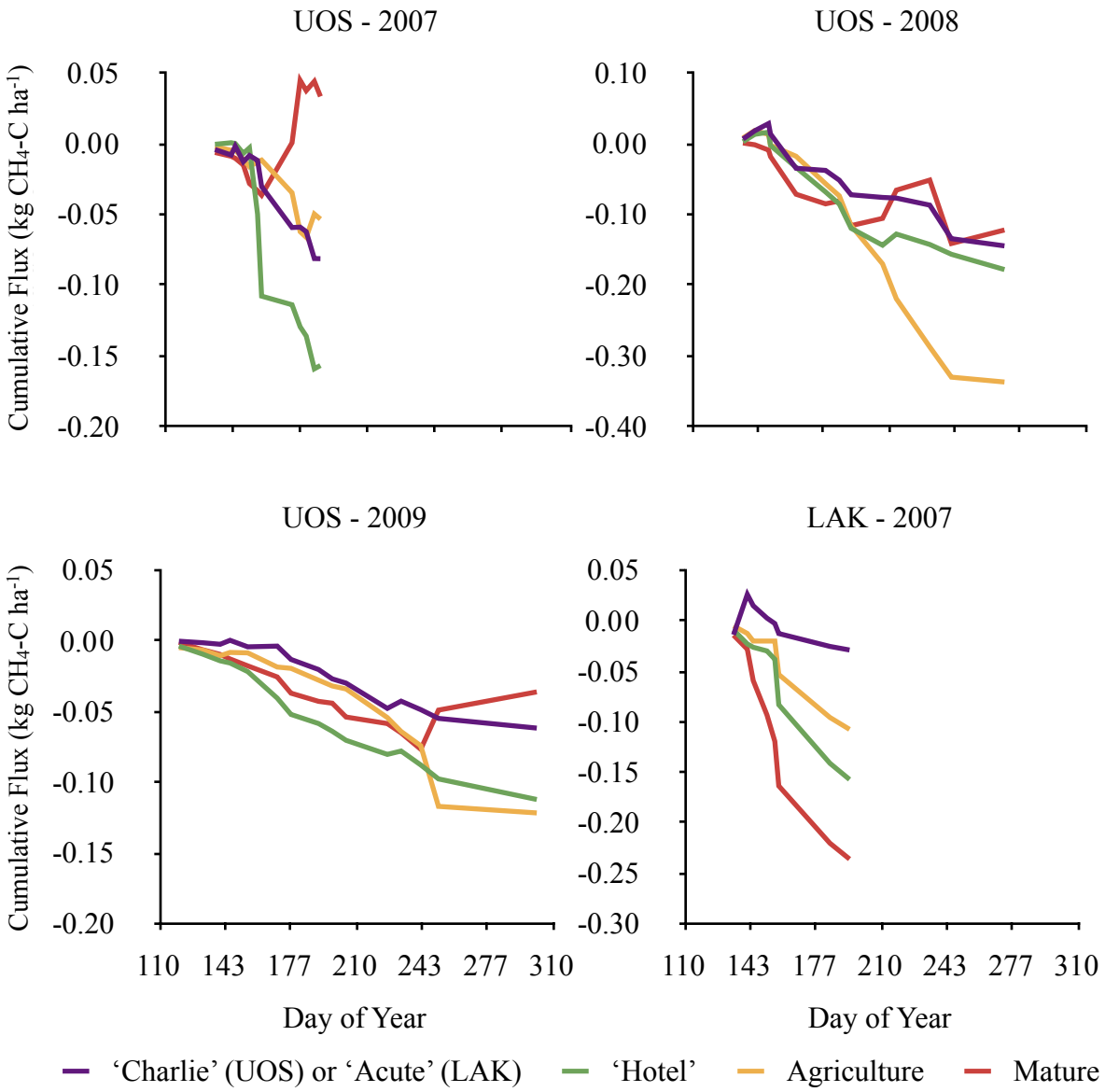

Figure 5.13: Cumulative methane fluxes from soil (kg CH_4-C ha^{-1}) at UOS and LAK sites for two clones of willow ('Acute' or 'Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

5.4.4 Carbon dioxide

Higher CO_2 efflux rates coincided with higher productivity at the different sites—this having a large influence on the differences seen between treatments. At the UOS site, CO_2 efflux

was initially highest in the agricultural soils and lowest in the plantation soils ('Charlie' in 2007, 2008, $P < 0.05$; 'Hotel' in 2008, $P < 0.05$). At this site willow productivity was low (5.3 Mg ha^{-1}). Over time CO_2 efflux increased in the UOS plantation as the trees became more established ,thereby negating any difference in 2009 (Table 5.3). However, the lower producing 'Hotel' clone still did not display the same pattern as the 'Charlie' clone or agriculture treatment (Figures 5.14-5.16). This is indicative of increasing root respiration as the trees grow and their root systems develop. The LAK willow had much more standing biomass than the UOS willow and therefore can be assumed to also have greater below ground biomass and consequently more root respiration. As well, the crop at the LAK site fared poorly and thus it had the lower soil respiration rates than the established forest whereas the willow did not ($P < 0.05$). Thus soil respiration rates appear to be tied more to plant growth than solely land use (Figures 5.14-5.17).

Soil respiration annually increased sooner in treed treatments as best seen in 2009 at UOS and 2007 at LAK (Figure 5.18). This is presumably because, being perennial, the trees are able to be active earlier in the growing season, including root respiration. This effect was not as readily seen in 2007 and 2008 willow at UOS due to their low productivity and young age.

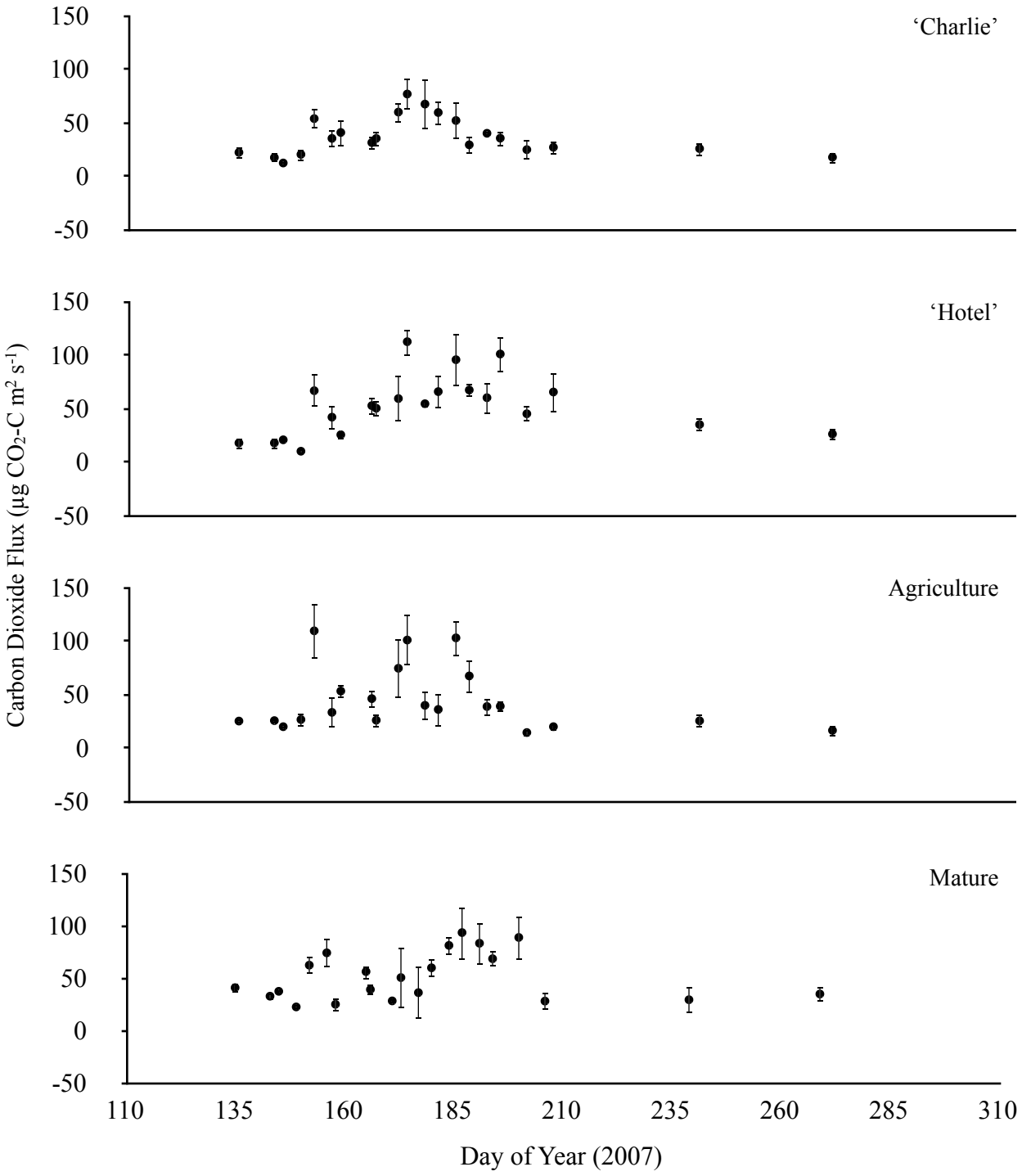

Figure 5.14: Carbon dioxide fluxes from soil (µg CO_2-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for UOS in 2007 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

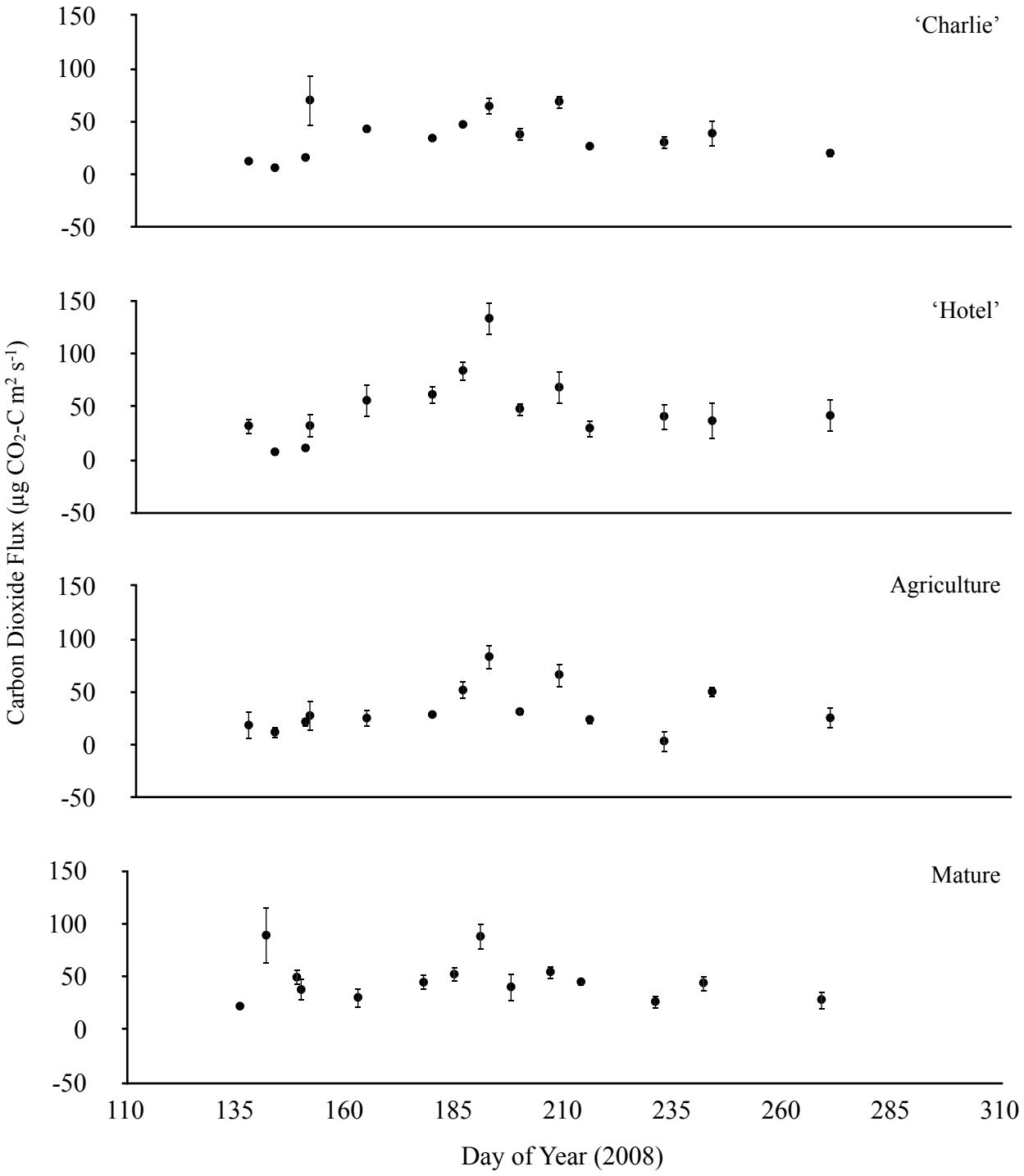

Figure 5.15: Carbon dioxide fluxes from soil (µg CO_2-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for UOS in 2008 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

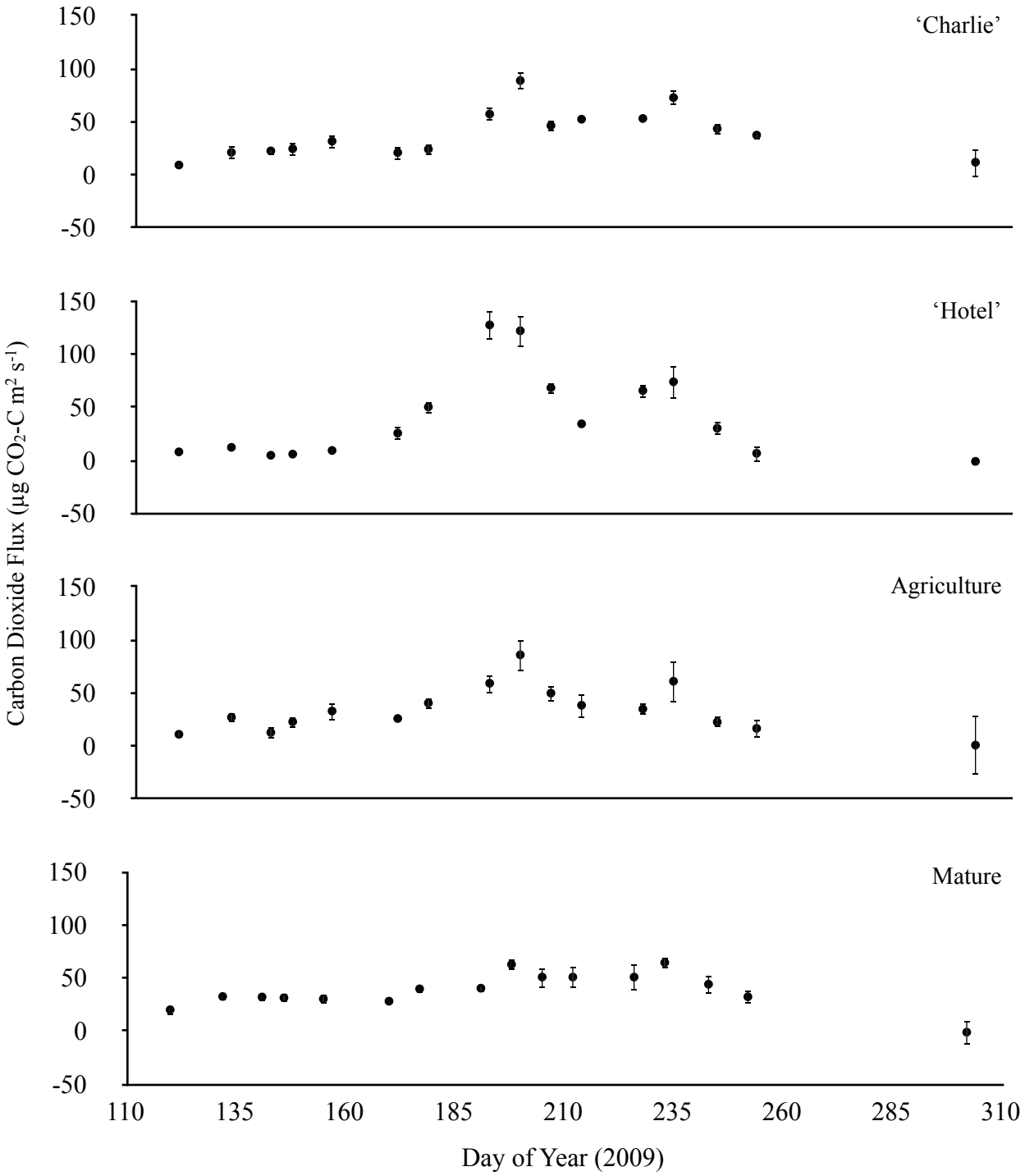

Figure 5.16: Carbon dioxide fluxes from soil (µg CO_2-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for UOS in 2009 for two clones of willow ('Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

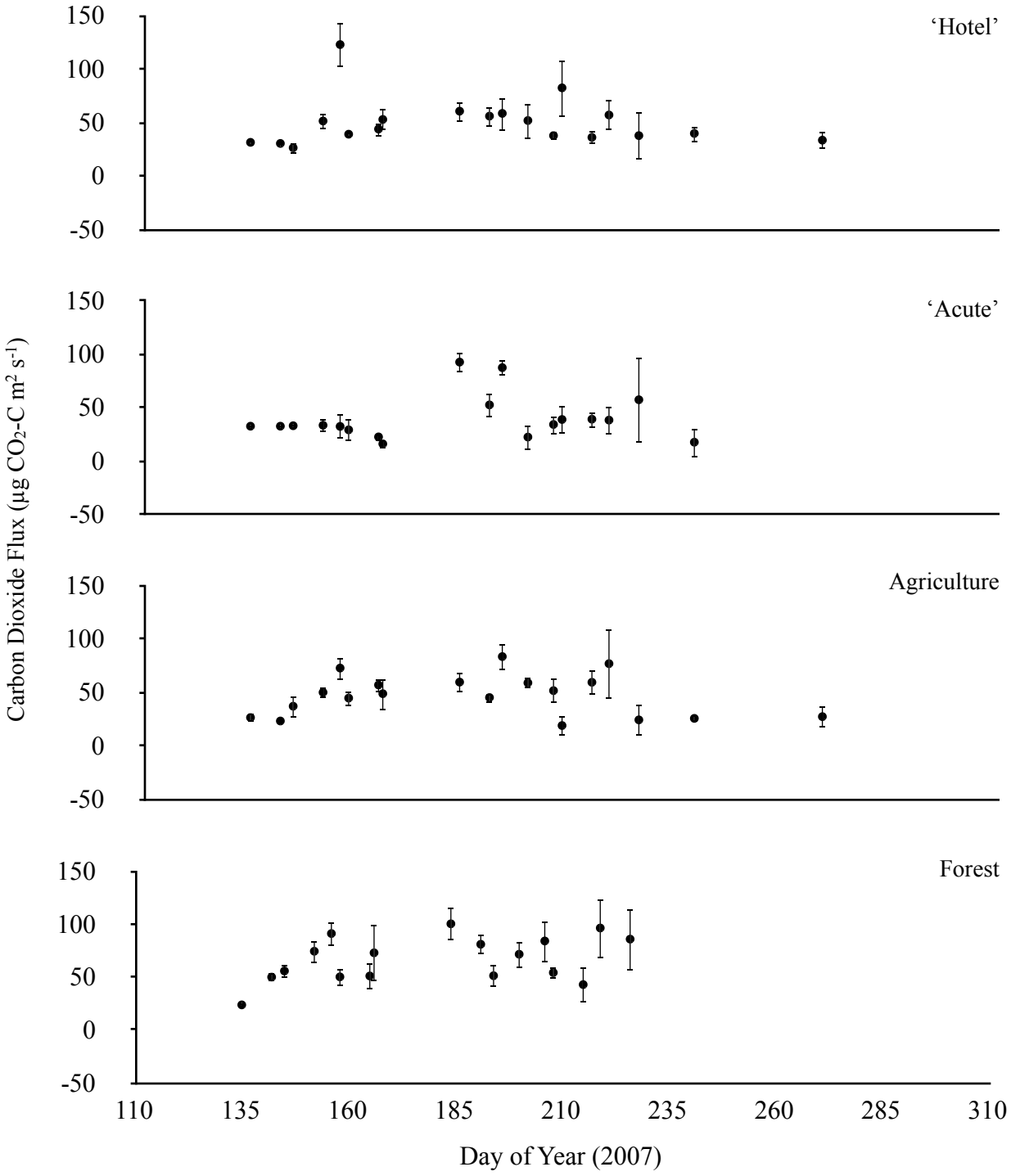

Figure 5.17: Carbon dioxide fluxes from soil (µg CO_2-C m^{-2} s^{-1}) (dots = measured emissions, error bars = standard error) for LAK in 2007 for two clones of willow ('Acute' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

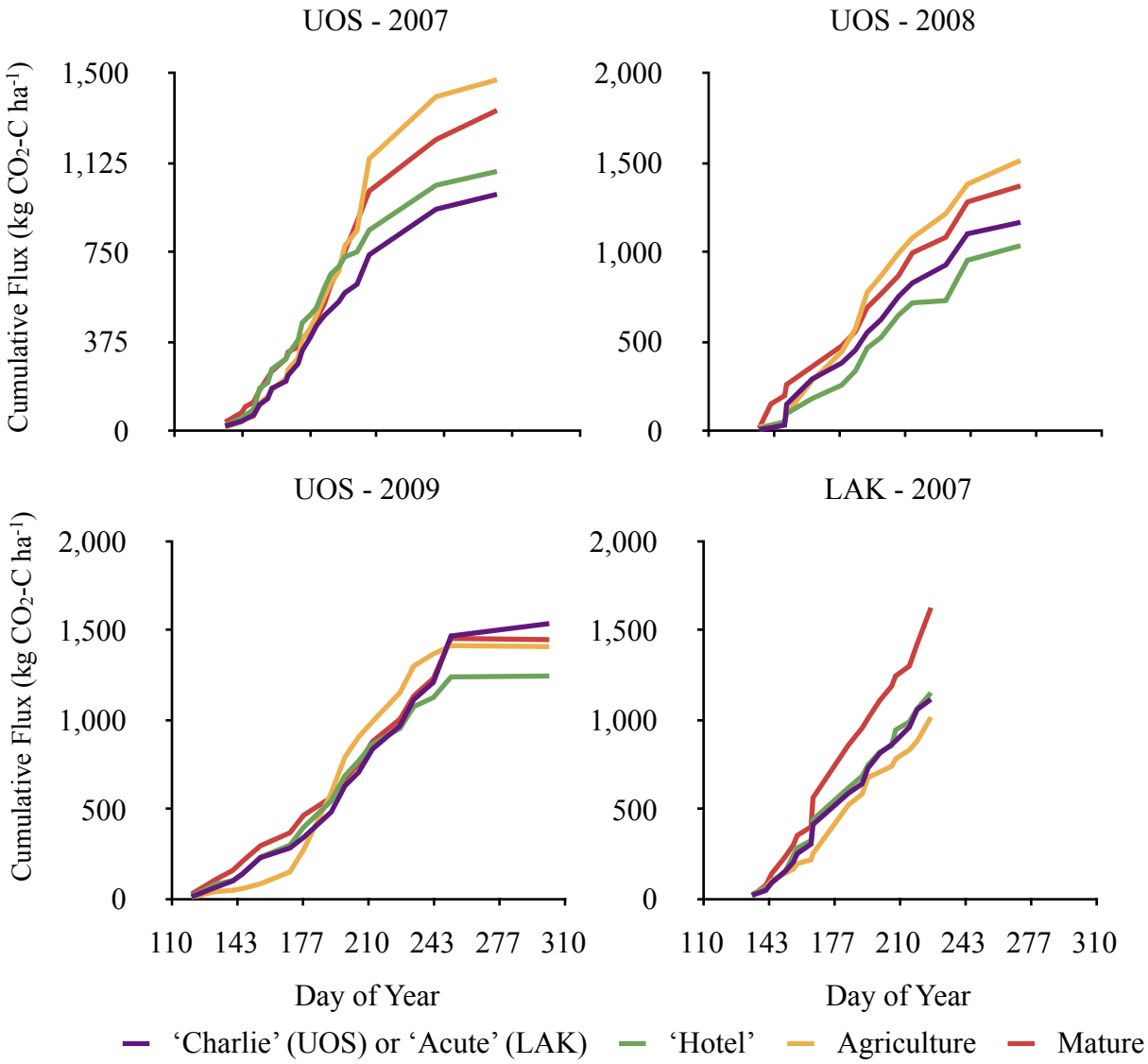

Figure 5.18: Cumulative CO_2 fluxes from soil (kg CO_2-C ha^{-1}) at UOS and LAK sites for two clones of willow ('Acute' or 'Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).

5.4.5 Changes in soil C

After the first three year rotation, soil C was on average depleted in nine willow plantations at 0-20 cm by a mean of -2.22 mg g^{-1}. This effect was most prominent when plantations were established where perennial species had previously been grown. This indicates that change in soil C is largely dependent on land-use history as described in Chapter 4.

Examining rates of soil CO_2 efflux compared to the change in soil C storage, loss in soil C through CO_2 efflux in the third through fourth year of growth (1.0-1.6 Mg ha^{-1} y^{-1}) does not account for the loss of C (mean of 5.5 Mg ha^{-1} across nine sites). Therefore the decrease in soil C is due to an initial establishment disturbance effect. Because root respiration increased with increasing aboveground productivity it can be assumed that root mass and fine root production and turnover also increased. Thus the potential to offset initial soil losses with accumulated biomass exists and the rate of accumulation is positively related to aboveground productivity. Therefore it can be assumed that there is a rapid loss of soil C associated with soil disturbance but that C will gradually accumulate until C saturation is reached or the site is disturbed again. There is strong support for this theory in the literature (Laganière et al., 2010). However, site quality has a large impact on C accumulation in aboveground biomass (Chapter 3) which varied from 2.6-11.2 Mg C ha^{-1} after three years of biomass accumulation with a mean of 4.5 Mg C ha^{-1} and can be expected to influence below ground biomass production as well.

5.5 Conclusions

The results indicate that a newly established low productivity willow plantation (1027-1103 CO_2eq-C plantation vs. 1554 CO_2eq-C agriculture, 2007 - UOS) produced lower GHG efflux than agricultural soils but as the willow grew this effect diminished (1400-1588 CO_2eq-C plantation vs. 1596 CO_2eq-C agriculture, 2007 - UOS). The different land uses respond differently to varying weather conditions from year to year making it difficult to say what the net effect will be on GHGs in the future without being able to predict future weather as well. However, some patterns do exist. There is a gradual shift in willow plantations from agricultural patterns of CO_2 and CH_4 efflux to that of mature stands with increasing cover (Figures 5.13, 5.18). Fluxes tended to be more consistent throughout the year under tree cover whereas under agricultural crops fluxes responded to the developmental stage of the crop and environmental conditions more readily. This shift occurs after an initial loss in soil C associated with land use change [-2.22 mg g^{-1} (Chapter 4)]. The presence of an initial C debt regardless of direction of land use change has been reported in the literature for other plantation systems (Guo and Gifford, 2002) and holds for willow plantations.

Previously derived C budgets for willow plantations have assumed that soil C will increaseor at minimum remain stable (Grogan and Matthews, 2002; Heller et al., 2003). However, land use conversion has a negative impact on soil C (Guo and Gifford, 2002). As such, C accounting in willow bioenergy plantations in temperate regions should take into consideration the initial C debt of conversion. The magnitude of this impact depends on the previous vegetation cover and management techniques employed. Conversion from heavily cultivated soils would be expected to have the smallest C debt as the soils are already depleted in soil C to varying degrees. Conversion from longstanding perennial land uses would have the greatest C debt particularly when litter layers are removed for site preparation. The incorporation in GHG budgets of decreased emissions of N_2O and increased consumption of CH_4 when compared to traditional agriculture is supported by this study in part as soil conditions (i.e. temperature, moisture and substrate) were altered towards what should result in a favorable soil GHG response. However, due to the sporadic nature of N_2O emissions, measurements of actual efflux were unable to detect a change in annual cumulative emissions.

Preface to Chapter 6

Chapters 3 - 5 examine willow productivity in terms of growth requirements and implications for soil nutrients and GHG balances. Productivity is not only a desirable goal for producers but as shown in Chapters 4 and 5 it also influences soil nutrient supply and GHG balances. However, the actual measurement of standing willow biomass has been difficult and time consuming. Therefore Chapter 6 develops and validates a new method for estimating standing willow biomass using a standard camera and readily available free software. Chapters 3 - 5 demonstrate that willow productivity should be monitored for achieving optimized yields currently and into the future as well as maximizing the benefit to GHG balances. Chapter 6 provides a reasonable method to accomplish a monitoring program non-destructively. The first manuscript in Chapter 6 describes the development of this method. The second manuscript in Chapter 6 is a follow up and validation of the first. It assesses the generalized applicability of the methods and models developed previously in the first manuscript.

6. DEVELOPMENT AND VALIDATION OF AN OPTICAL METHOD FOR ESTIMATING STANDING BIOMASS IN SHORT ROTATION INTENSIVE CULTURE

PART I: RAPID BIOMASS ESTIMATION USING OPTICAL STEM DENSITY OF WILLOW (*SALIX* SPP.) GROWN IN SHORT ROTATION

Part I of Chapter 6 has been previously published (with minor changes) as:

Ens, J.A., R.E. Farrell, and N. Bélanger. 2009. Rapid biomass estimation using optical stem density of willow (*Salix* spp.) grown in short rotation. Biomass Bioenerg 33:174-179.

6.I.1 Abstract

Quick and accurate biomass estimation of willow (*Salix* spp.) grown under short rotation intensive culture (SRIC) is essential for C accounting and management decisions. Currently, most estimates of tree biomass, including willow, rely on measurement of stem diameter. This is a suitable approach for single-stem species but for measurements of multi-stem species such as willow, there is an increase in the time and effort required as well as the need to include site, clone and age specific information. Therefore, I developed a new method which calculates optical stem density from digital photographs taken at predetermined locations and angles within a plantation during the fall or winter when the willow is without leaves. I then calibrated a mathematical model using destructive sampling to convert the measurements of optical stem density into estimates of biomass. The method produced very strong relationships (adjusted r^2 = 0.97) between the predicted and actual harvested biomass for the plots studied. Being new, the method still requires further testing and possibly adjustments for different planting designs and clones.

6.I.2 Introduction

Accurate biomass estimation of willow grown in short rotation is essential for C accounting and plantation management. Allometric relationships are commonly used for single

stem species and stem diameter or stem diameter and height can be used to accurately estimate the biomass of various tree components by simply modifying coefficients of standardized equations for different species (Lambert et al., 2005). However, willow presents several challenges not seen with other tree species. In SRIC, the multi-stem nature of willow means that this process must be conducted on a sampling area containing many stems (Verwijst and Telenius, 1999), thus becoming more time consuming and introducing increased error and more opportunity for bias than with single-stem trees. Nevertheless, the best estimates of willow biomass largely rely on this approach (Verwijst and Telenius, 1999; Arevalo et al., 2007). Allometric equations must also be adapted for tree age and each individual clonal variety to yield a high enough level of accuracy (Verwijst and Telenius, 1999; Arevalo et al., 2007). This becomes especially difficult for plantations that contain multiple varieties or intercropped varieties and/or species (Norman and Campbell, 1989). Additionally, this method has a tendency to overestimate biomass when the stems are intact and healthy at the base but are damaged or destroyed higher up (Verwijst and Telenius, 1999). Therefore, there is a need to explore other approaches to estimate biomass in SRIC of willow. The ideal method is non-destructive, inexpensive, quick, independent of clonal variety and accurate.

Optical measurements have been used extensively for characterizing and measuring various tree components, stand density and canopy architecture for forestry and other applications. Light dynamics and canopy cover have been described using numerous devices including the spherical densiometer, leaf area index meter (LAI-2000; Li-Cor Biosciences, Lincoln, NB), horizontoscope and the moosehorn (Lieffers et al., 1999). These same measurements of light transmission or reflectance of various spectra have proven to be effective in quantifying components of canopy structure such as leaf area index (Norman and Campbell, 1989). Photography is one method that has been valuable for making these types of measurements (Rich, 1990; Guevara-Escobar et al., 2005). Photographic analysis is not limited to quantifying the interactions between trees and light. For example, Heisler and Dewalle (1988) were able to calculate windbreak effectiveness from photographs of relatively narrow shelterbelts.

The effectiveness of visual estimation for these applications suggests that it may also be possible for visual estimation to be an effective means of measuring biomass in highly complex willow plantations. Ter-Mikaelian and Parker (2000) used photography to produce accurate biomass estimates of white pine (*Pinus strobus* L.) by calculating the area, within a photograph, of a tree's silhouette against a screen. Although this method yielded good results ($r^2 = 0.98$ for aboveground biomass), it was noted that it could not be used after canopy closure. The method as developed by Ter-Mikaelian and Parker (2000) would not be practical or even possible in high density willow plantations because there is insufficient tree spacing for the use of a screen (i.e., for side-looking photographs to be taken) and to isolate individual trees or plots. Although this particular method cannot be used in willow plantations, it does illustrate the potential of photography for such measurements. Optical measurements derive information from a much greater percentage of the total volume of the trees than point source measurements of stem diameter and as such have the potential to make use of more accurate and more transferable allometric relationships. Therefore, an indirect method for biomass estimation in willow plantations using optical measurements is worth pursuing. This is echoed by Norman and Campbell (1989, p. 303) who stated that when making measurements of canopy structure, "the advantages of indirect are so overwhelming that in the future direct methods will only be used when there is no indirect alternative."

The goal of this study is to assess the potential of optical stem density measurements in developing a method for accurately estimating biomass of willow grown in SRIC. This includes both an assessment of the strength of stem density to biomass relationships using optical stem density as well as identifying areas for improvement.

6.I.3 Materials and Methods

Three sites were selected across the Canadian prairies to provide a range of growing conditions for a single willow variety, *S. purpurea* 'Hotel' (Table 6.1).

Table 6.1: Site and productivity information for 3 year old *S. purpurea* 'Hotel' clones.

Location	Latitude	Longitude	Soil	Mean Annual Precipitation†	Degree Days (Above 5°C)†	Productivity (Dry Weight)‡
						Mg ha^{-1}
Smoky Lake, AB	54.09° N	112.25° W	Sand	374 mm	1406	5.12 – 5.82
Lakeshore, SK	51.99° N	106.77° W	Silty loam	350 mm	1644	14.84 – 21.84
Portage la Prairie, MB	49.96° N	98.17° W	Clay loam	515 mm	1784	13.83 – 33.09

† Climate average for the last 30 years (Environment Canada, 2008).
‡ Total after three years of growth.

All three sites were planted in 2005 and used the same spacing. Trees were planted in north-south oriented triple rows with 60 cm between trees and 200 cm between each triple row. At each site, five plots (each 180 cm by 320 cm) were established to incorporate an area that contained a combination of row and aisle areas proportional to the total amounts in the plantation. Based on the original planting design (Fig. 6.1), each plot included the bases of nine trees. Due to mortality, however, not all plots contained nine living trees.

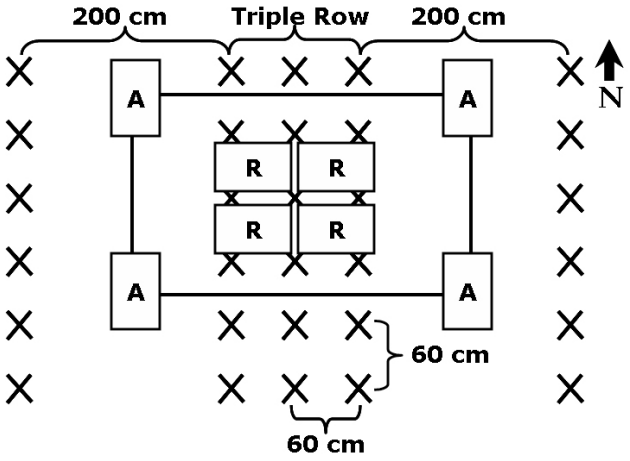

Figure 6.1: Planting and imaging layout and orientation (A = aisle, R = row, X = willow, box = imaging point of view).

In this study, I developed a procedure that can be used to rapidly and accurately assess biomass in willow plantations immediately preceding harvest. The optimal time to harvest willow is after leaf senescence, both to improve nutrient cycling by allowing leaves to decompose on site and to ensure the health of the trees that is maintained for subsequent rotations (Ranger and Nys, 1996). Therefore, the optimal period for accurately estimating accumulated and harvestable biomass, either for C accounting or management decisions, is immediately after leaf senescence. This is fortuitous as it also allows a view of the stems that is unobstructed by leaves at the time of measurement. However, as this study took place in October and leaf senescence had not yet fully occurred, all remaining leaves were stripped from the trees and all weeds were removed to simulate the proper conditions.

Once the proper conditions were met, the following procedure was used to determine the potential of optical stem density for estimating biomass. Images were taken of each plot after which all stems originating in the plot were harvested by hand at ground level and weighed with a scale in the field. Representative samples of the stem biomass from each plot (lower, mid and top stems and shoots) were collected, taken back to the laboratory, and dried to a constant weight at 35°C for 12 days. The calculated percent moisture of these samples was then used to convert the fresh weight of the total harvested biomass of each plot to dry weight.

Digital photographs of the trees were taken using a Canon EOS Digital Rebel camera fitted with the standard EF-S 18-55 mm lens. Photographs were taken vertically such that the willows were the foreground and the sky the background. The EF-S 18-55 mm lens is designed specifically for the Canon EOS Digital Rebel Camera and when used on this camera has an angle of view equivalent to that of a 29-88 mm lens on a 35 mm camera (Canon Canada Inc., 2008). The focus was adjusted manually so that the depth of field included the majority of the stand and the lens was set at 28 mm (45 mm on a 35 mm camera), which provided an angle of view of approximately 44° on the horizontal axis of the image and 30° on the vertical. This is opposed to a more conventional fish-eye lens, which have a very large angle of view (sometimes exceeding 180°) and is typically used for other visual assessments of canopy structure (Rich, 1990). While fish-eye lenses have the ability to capture nearly all the stems within the plot, the lens was set at

28 mm as a trade-off between imaging the maximum amount of stem area from within the plot and minimizing the amount of stem area from outside the plot (Fig. 6.2).

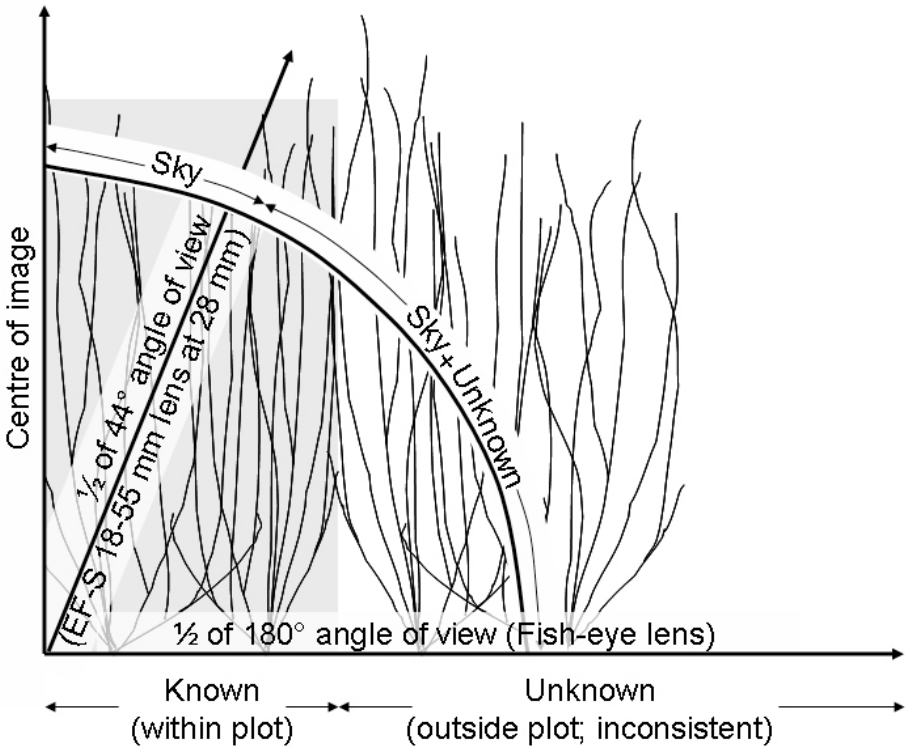

Figure 6.2: Comparison of EF-S 18-55 mm lens to Fish-eye lens for application in a willow plantation. Shading represents within plot volume.

A total of 16 images (grouped into four sets of four images according to their points of origin) were taken for each plot. Images were taken from four points within the row and from the aisle at the four corners of the plot (Fig. 6.1). This was done to capture a range of stem densities for each plot. At each of these points, images were taken with the focal point of the camera at 8 cm and 100 cm above ground. The height of 8 cm was selected as the lowest position possible given the constraints of operating the camera. The height of 100 cm was selected based on preliminary tests that suggested that a 100 cm height provided a large change in optical density from 8 cm while at the same time capturing as much of the stems as possible. Therefore, the four sets of images taken in each plot included: four images from inside the row at 8 cm above ground level (R_8), four images from inside the row at 100 cm above ground level (R_{100}), four images

from the aisle at 8 cm above ground level (A_8) and four images from the aisle at 100 cm above ground level (A_{100}). Images taken from the aisles were cropped such that only the quarter of the image (i.e., 90°) which angled toward the plot remained.

The program Scion Image, Beta 4.0.3 (Scion Corporation, Frederick, Maryland) was used to calculate optical stem density for each image because of its ability to (i) automatically calculate threshold values between foreground and background and (ii) make area measurements. The first step for calculating optical stem density from the image was to calculate and record the total number of pixels in the image. The values of the image corresponding to the blue portion of the spectrum were then selected as they produced the highest contrast between foreground and background over a variety of sky conditions. The next step was to choose a threshold value so that all pixels with values greater than the threshold were included and all pixels with values lower than the threshold were excluded. The stems were all included as they had greater values than the sky (Fig. 6.3). This can be done automatically or manually with Scion Image. For the sake of simplicity and rapidity the thresholds were calculated automatically unless errors were visually observed.

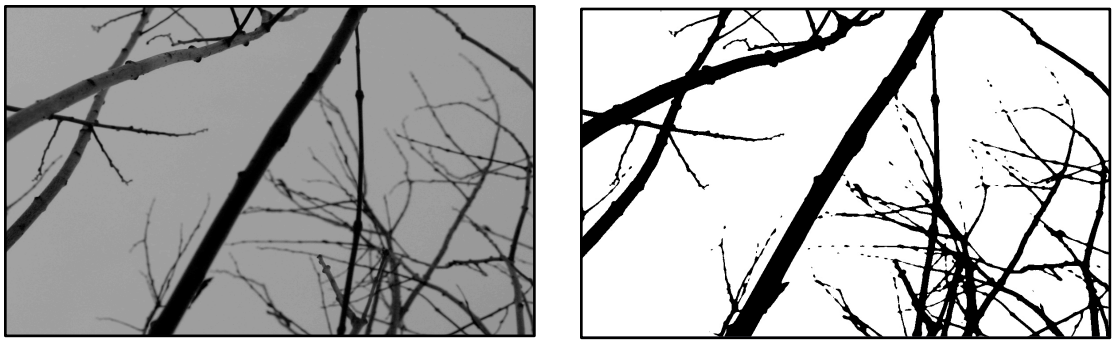

Figure 6.3: Selecting stemwood from the raw image (left) by selecting pixels above threshold (right).

Sources of error in the field include the positioning and focus of the camera and the environmental conditions. Partially cloudy conditions and poor focusing (caused by wind and movement of the stems) can cause minor problems with the threshold which can largely be corrected in the image processing phase. Most of the processing can be done automatically, but

there are some pictures that require manual adjustment of the threshold level. Such cases are quite manageable, which means that the method will yield consistent and accurate results despite these problems. In cases where variations in cloud cover caused the threshold to include part of the sky, the threshold value was manually increased (darkened) until none of the background was included with the stems (Fig. 6.4).

Figure 6.4: Comparison of raw image (left) to pixels selected by automated threshold (centre) and pixels selected by manual threshold (right) with inconsistent sky conditions.

Manual adjustment of the threshold also was needed in cases where a low branch caused the image to be out of focus, thereby disproportionably increasing the number of pixels above the threshold. In these instances, the threshold was again increased until the area included more closely fitted the actual branch size (Fig. 6.5).

Figure 6.5: Comparison of raw image (left) to pixels selected by automated threshold (centre) and pixels selected by manual threshold (right) with poorly focused image.

Wind can also generate variability in optical stem density measurement as it affects the angle of the branches and thus modifies the surface area in the image. Similarly, should the camera be poorly positioned, it is obvious that the measured surface area will differ from that of the targeted stems. However, thus far, this method has proven to be quite robust to slight imperfections in

camera positioning and wind disturbance in the field. Finally, it is assumed with this method that the surface area of branches moving out of the image is the same as that of the branches moving into the image. On average the assumption that branching in equals to branching out should be true and so the effect of this error should be minimal.

Once the threshold was established, the number of pixels with values greater than the threshold was counted and recorded. The number of pixels with values greater than the threshold (P_{thr}) was divided by the total number of pixels (P_{tot}) and multiplied by 100 to yield the optical stem density (OSD) as a percentage:

$$OSD = (P_{thr} / P_{tot}) \times 100 \quad [6.1]$$

Once the optical stem density was calculated for each individual image, the values were averaged for each set of four images. Thus, for each plot one data point was calculated for R_8, R_{100}, A_8 and A_{100}.

The average optical stem density for each point of origin (i.e., R_8, R_{100}, A_8 and A_{100}) was plotted against the calculated dry weight and regression analysis was conducted using SPSS 15.0 for Windows (SPSS, Chicago, Illinois). Curves were fitted to the data using a series of models and assessed for goodness of fit using the coefficient of determination. A stepwise multiple linear regression was then computed, with all points of origin as possible input variables, to determine if a better model could be produced relative to the models with only one point of origin.

6.I.4 Results and Discussion

The optical stem density calculated from all four points of origin showed strong relationships to harvested biomass (Table 6.2; Fig. 6.6). The highest r^2 values were obtained with A_8 and R_{100}. In all cases, the quadratic model produced the highest r^2 values. However, for R_{100} and A_{100}, there was very little improvement in the predictive capability of the quadratic model relative to the linear model. Student's t-tests ($P < 0.10$) confirmed that the quadratic term was significant for R_8 and A_8 but not significant for R_{100} and A_{100}. Therefore, quadratic models were chosen for A_8 and R_8 while linear models were considered adequate to explain R_{100} and A_{100}. The

stepwise multiple linear regression model produced from using A8 and R_{100} as input variables improved predictions of harvested biomass (Table 6.2; Fig. 6.7).

Table 6.2: Models and equations used in regression analysis and the strength of the relationship described.

Point of origin	Model	Equation†	Adjusted r^2
R_8	Quadratic	$\hat{y} = -0.025R_8^2 + 2.46R_8 - 27.16$	0.87***
R_{100}	Linear	$\hat{y} = 1.13R_{100} + 2.33$	0.92***
A_8	Quadratic	$\hat{y} = -0.018A_8^2 + 1.56A_8 + 4.65$	0.93***
A_{100}	Linear	$\hat{y} = 2.41A_{100} + 7.05$	0.86***
Multiple regression	Linear	$\hat{y} = 0.599R_{100} + 0.597A_8 + 3.48$	0.97***

† All terms of the equations are significant at $P < 0.10$
*** All models are significant at $P < 0.001$

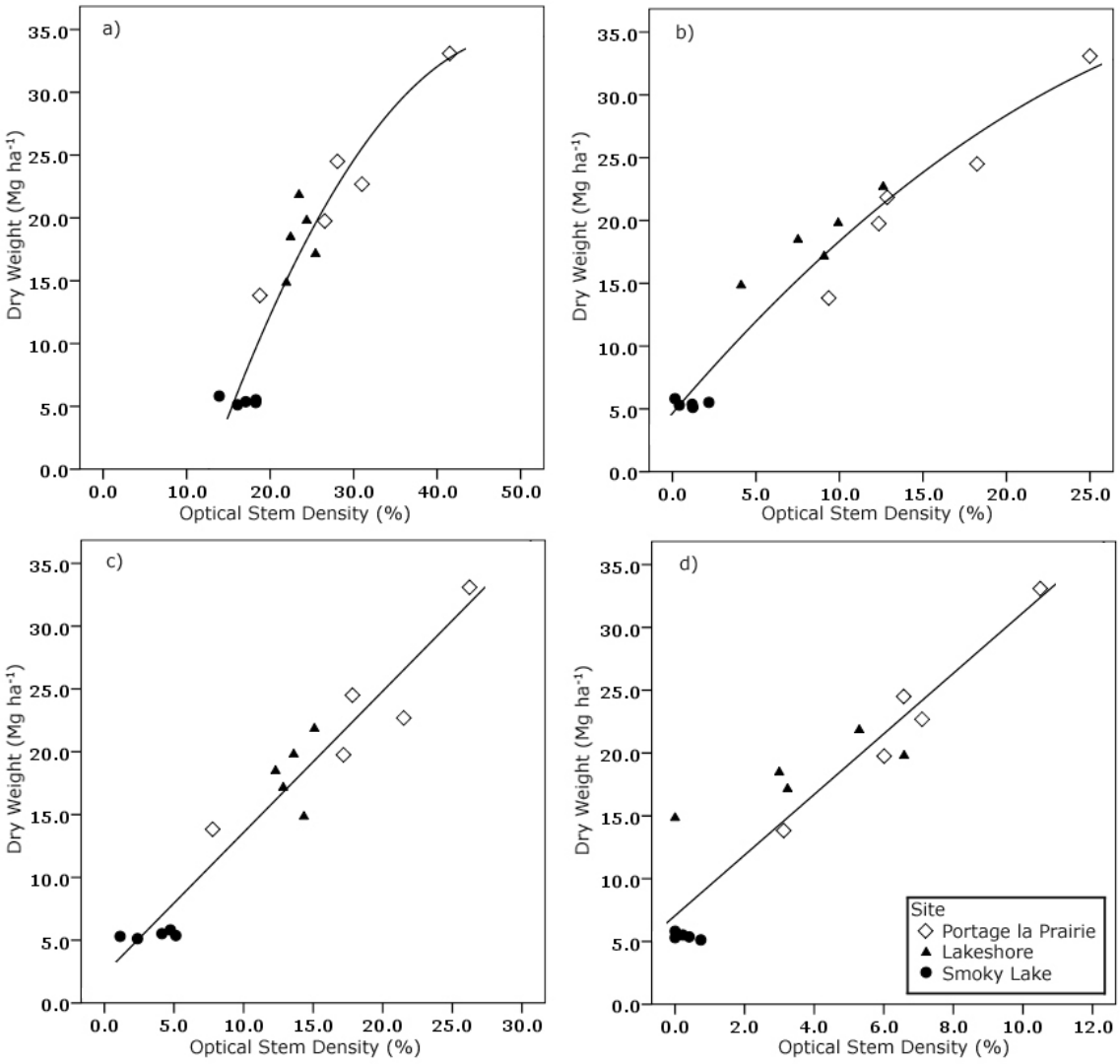

Figure 6.6: Regression analysis plots for models including a single point of origin from R_8 (a), A_8 (b), R_{100} (c), and A_{100} (d).

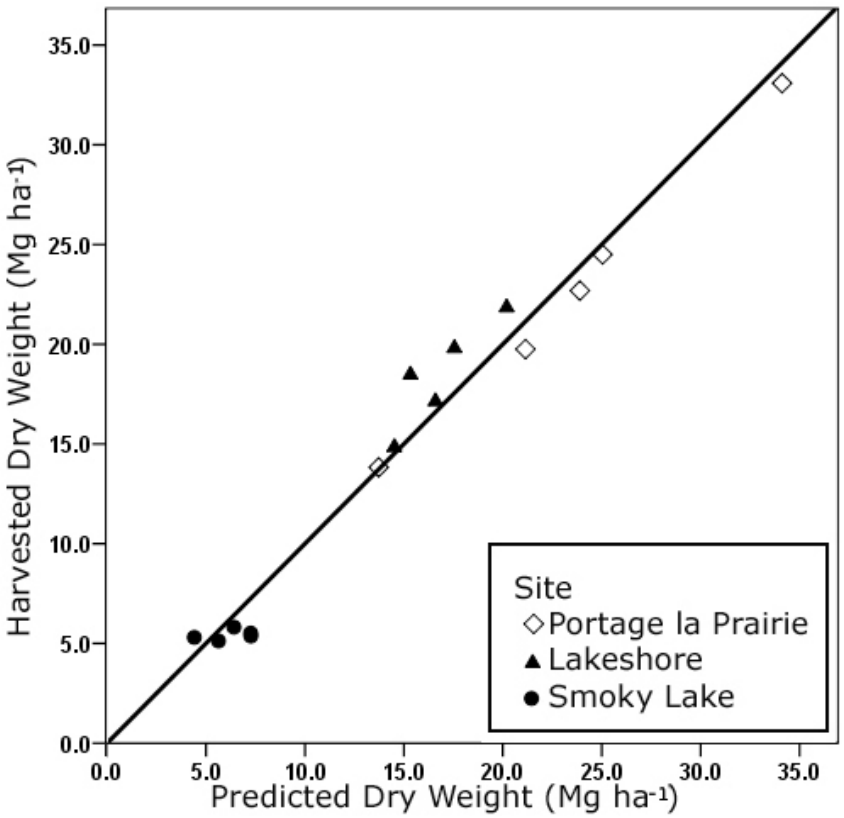

Figure 6.7: Predicted biomass by optical stem density using model derived from multiple linear regression vs. actual harvested biomass against 1:1 line.

Due to the angle of view of the camera, horizontal branching produced a disproportionately large area on the images compared to vertical branches. This effect, most prominently seen in R_8 (Fig. 6.6a), was responsible for the curving of the regression lines. Judging from the regression analyses it can be said that factors controlling optical stem density were not the same for different points of origin. This can be explained by the general morphology of the 'Hotel' clone. At its base, 'Hotel' spreads laterally and much of the branching occurs near the ground. After branching from the main stems, the branches curve upwards and grow near vertical due to intraspecific competition for light. As competition for light increases, apical growth becomes more dominant (Kozlowski et al., 1991). Consequently, changes in morphology as a result of competition result in a lower proportion of horizontal branching with increasing productivity, distance from the ground and distance from the row centre. Thus, the

greatest effect of horizontal branching from R_8 followed by A_8 and the least from A_{100} and R_{100}. A visual comparison of images confirmed that horizontal branching was indeed creating disproportionately large surface areas in the R_8 images of the Smoky Lake plots and a few lower productivity plots at Portage la Prairie and Lakeshore.

For the R_8 set of images, the strong curvilinear line with an intercept substantially different from zero (Fig. 6.6a) associated with the effects of horizontal branching is likely the cause for the omission of this point of origin in the multiple linear model. Conversely, although it showed no evidence of effects from horizontal branching, A_{100} images had the weakest relationship to harvested biomass ($r^2 = 0.86$). This probably reflects the fact that there was not enough surface area (or optical stem density) captured in the images to make accurate predictions of harvested biomass. Therefore, there is a trade-off between capturing the most optical stem density possible and minimizing the effect of horizontal branching (i.e. R_8 images). For the 'Hotel' clone, it appears that the A_8 and R_{100} images provide the best balance and when combined in a multiple linear model provide very accurate biomass estimates (adjusted $r^2 = 0.97$). As the combination of A_8 and R_{100} images made use of measurements from both the aisle and the row as well as from both 8 cm and 100 cm, they were able to capture most of the variability within the plot without encountering the extra unexplained variability seen in A_{100} and R_8. Knowing that this trade-off exists means that further experimentation with different points of origin could lead to even more accurate biomass estimations. At first glance, it would seem that R_8 images are not likely to be useful in predicting biomass. However, because R_8 images are affected by morphology to a far greater extent than other points of origin, it may prove to be an important image type for other clones. In this way, optical stem density measurements may prove to be adaptable to a variety of willow clones with distinct morphologies.

The optical stem density method described here is considered to be site independent. The three sites chosen include a wide range of environmental and growing conditions along a 1000 km transect across the Canadian prairies. The Smoky Lake site had very little standing biomass, whereas the Lakeshore and Portage la Prairie sites had much higher values and a large range of biomass density. Yet, when all the sites are included in the multiple linear regression, all

predicted values fall very close to the actual harvested biomass (Fig. 6.7). This strongly suggests that the stem density to biomass model built for the 'Hotel' clone is independent of site.

The accuracy of the optical stem density method is very high for the plots tested in this study. Therefore biomass estimation via optical stem density is an accurate method for quickly and non-destructively assessing biomass of willow under SRIC. Adjustments may nevertheless be necessary for different clones and planting designs and thus, further testing of the approach will be necessary.

PART II: VALIDATION OF THE OPTICAL STEM DENSITY METHOD FOR RAPID BIOMASS ESTIMATION IN SHORT ROTATION INTENSIVE CULTURE OF WILLOW

6.II.1 Abstract

Accurate non-destructive estimates of standing biomass in short rotation intensive culture of willow are important for management purposes including determining optimal rotation length and C accounting. The optical stem density method developed by Ens et al. (2009) provided a promising approach. This method was validated with a new data set derived from four new sites. Each site had the same willow clone (*S. purpurea* 'Hotel') and planting design as the original data set. Coefficients of determination were lower (maximum of $r^2 = 0.80$) during validation then in development (maximum of $r^2 = 0.97$). Lower coefficients of determination were largely due to a wider range in growth habits expressed at the different sites and a smaller range in productivity overall. R_{100} was the only point of view that stood up reasonably well to validation. Optical stem density continues to be a promising measure of standing biomass for management purposes as accurate measures on a plantation scale can be made by increasing the number of plots sampled.

6.II.2 Introduction

Estimating standing biomass non-destructively in short rotation intensive culture (SRIC) of multi-stemmed species such as *Salix* and *Populus* when coppiced is difficult. There is no single stem at breast height with which to form a basis for allometric equations, though there

have been numerous attempts made to develop a suitable method of measurement (Arevalo et al., 2007; Verwijst and Telenius, 1999). Thus far, biomass estimation based on allometric equations requires multiple measurements and calibration for both species and site (Arevalo et al., 2007; Verwijst and Telenius, 1999). The differing growth habits that can be attributed to different soil and climatic conditions are enough to cause divergence in equations from site to site, even within the same species or clone.

The optical stem density method, developed by Ens et al. (2009), provides a potential means for quick and accurate measurement of standing biomass in high density multi-stem tree plantations. The method yielded strong relationships (adjusted r^2 as high as 0.97) between standing biomass and optical stem density, which is defined as the percentage of an image comprised of stems when taken facing upwards. Ens et al. (2009) developed the optical stem density method using three sites (Table 6.3), two of which showed good interspersion of standing biomass. However, at the time, there was no independent data set available to test the applicability to other plantations. It is hypothesized that the optical stem density method is a robust measure of willow biomass since it makes use of the entire length of stems in the measurement. This was tested using an independent dataset incorporating four new sites with willow grown in SRIC.

Table 6.3: Site locations and standing biomass after three years of growth.

ID	Geographic Coordinates	Harvested Biomass
		Mg ha^{-1}
Original dataset		
Smoky Lake, AB (SMO)	54°07'N 112°24'W	5.1 - 5.8
Lakeshore Tree Nursery, Saskatoon, SK (LAK)	52°00'N 106°45'W	14.8 - 21.8
Portage la Prairie, MB (POR)	49°57'N 98°10'W	13.8 - 33.1
Validation dataset		
University of Saskatchewan, Saskatoon, SK (UOS)	52°07'N 106°36'W	3.4 - 7.8
Sault Ste. Marie, ON (SSM)	46°32'N 84°24'W	5.2 - 6.9
Guelph, ON (GUE)	43°33'N 80°13'W	4.4 - 18.3
Pickering, ON (PIC)	44°00'N 79°01'W	6.8 - 17.6

6.II.3 Materials and Methods

Following the optical method and using the equations developed by Ens et al. (2009), standing biomass was estimated at four independent sites (Table 6.3) with the same willow clone and planting design as in the previous experiment. Biomass originating within the plot was then harvested, weighed and dried to give the actual standing biomass at any given plot.

Digital images were captured at four points of view and replicated four times for any given plot. The points of view were in the willow row itself at 8 cm (R_8) and 100 cm (R_{100}) above ground height as well as the aisle between the rows again at 8 cm (A_8) and 100 cm (A_{100}). Images were taken with the camera pointing straight up such that the stems were the foreground and the sky was the background. Images were processed for the percent of the image covered by stems. The mean of the four replicates was taken as the optical stem density of the plot for that specific point of view.

Standing biomass was estimated using the equations from Ens et al. (2009) as well as new equations developed with the new dataset and the combined dataset for comparison. Statistical analyses were conducted with CoStat (CoHort Software, Monterey, CA).

6.II.4 Results and Discussion

The original empirically derived linear and quadratic models from Ens et al. (2009) had very high adjusted r^2 values (i.e., > 0.90). However, when used on the validation dataset, r^2 dropped substantially (Table 6.4). This is expected, as regression tends to produce optimistic equations if only applied to the data used for their development (Snee, 1977). For the validation set, the model derived by multiple linear regression incorporating both A_8 and R_{100} (Figure 6.8) fared worse than that with the R_{100} point of view only (Figure 6.9). Despite expectations of lower coefficients of determination, the substantial drop in r^2 is somewhat disappointing. Some of the decline in r^2 can be attributed to the smaller range in the validation dataset which does not include highly productive sites (~30 Mg ha^{-1}). The cluster of data points at the low end of productivity inherently had a lower r^2 even though they were in relatively good agreement with

the 1:1 predicted vs. harvested line. While the R_{100} point of view proved to be reasonably robust, the other points of view did not stand up well to validation (Table 6.4).

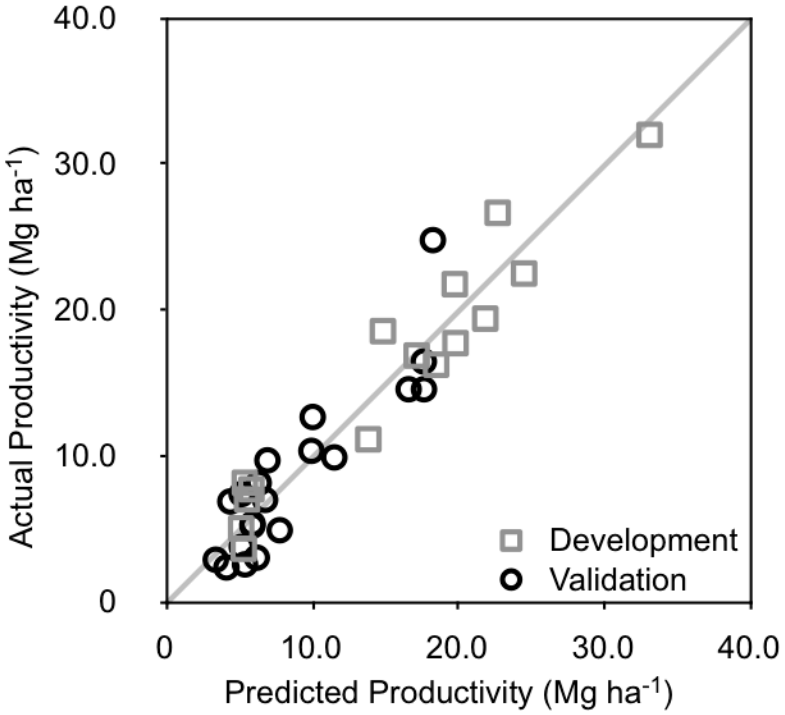

Figure 6.8: Predicted, using multiple linear regression model from Ens et al. (2009) (Equation 6 in Table 6.2), versus actual harvested biomass. Development and validation data shown against 1:1 line.

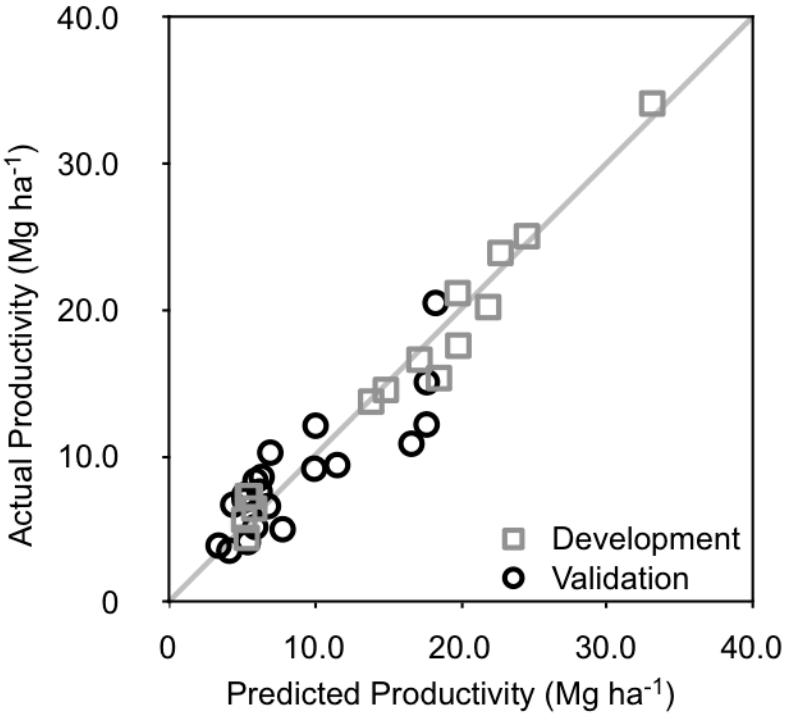

Figure 6.9: Predicted, using R_{100} model from Ens et al. (2009) (Equation 4 in Table 6.2), versus actual harvested biomass. Development and validation data shown against 1:1 line.

Table 6.4: Comparison of regression models for predicting standing biomass from optical stem density using the validation data set only and a combined full data set. Empirical models were used to predict standing biomass for the development data set (original data from Ens et al. (2009)), the validation data set (new sites in this study used to test the models) and the combined dataset (development and validation datasets as one). Models tested include those from Ens et al. (2009) (Original) and those developed using the combined data set (Combined).

Model	Equation	Data	Adjusted r^2
1. Original A_8	$y = -0.018\ A_8^2 + 1.56\ A_8 + 4.65$	Development	0.93
		Validation	0.20
2. Original A_{100}	$y = 2.41\ A_{100} + 7.05$	Development	0.86
		Validation	-0.05
3. Original R_8	$y = -0.012\ R_8^2 + 2.46\ R_8 - 27.16$	Development	0.87
		Validation	-0.02
4. Original R_{100}	$y = 1.13\ R_{100} + 2.33$	Development	0.92
		Validation	0.80
		Combined	0.90
5. Combined R_{100}	$y = 0.88\ R_{100} + 3.89$	Development	0.92
		Validation	0.80
		Combined	0.90
6. Original Multiple Linear Regression	$y = 0.599\ R_{100} + 0.597\ A_8 + 3.48$	Development	0.97
		Validation	0.66
		Combined	0.90
7. Combined Multiple Linear Regression	$y = 3.23\ R_{100} + 3.42\ A_8 + 0.799$	Development	0.92
		Validation	0.28
		Combined	0.95

The larger divergences from the poorer models can be attributed to differing growth habits within the same clone. There were two main variations in growth habit noted across the sites despite that all willows were of the same clone. The first is the amount of sprawl at the base of the willow. Ens et al. (2009) initially attributed this phenomenon to the poorer producing plots but examination of the validation sites shows this to be more of a site specific variation, regardless of productivity. The main stem of the willow may grow along the ground (often rooting along the way) and produce upright secondary stems branching perpendicular to the main stem or it may grow more upright at its base and secondary stems grow upright branches at acute angles. This difference accounts for the poor performance of the R_8 point of view as there is substantial variability near the camera that is not necessarily proportional to the overall productivity of the plot. The second divergence in growth habit is the degree of canopy closure into the aisle space. This had a much greater effect on low productivity plots where willow branching could be completely absent from the space between rows. This variability greatly decreases the effectiveness of the A_8 and A_{100} points of view when the presence or absence of branches was determined more by growth habit than by correlation with total standing biomass.

The R_{100} point of view is located where such differences are minimized. The model based on R_{100} exclusively therefore had the smallest drop in r^2. The strength of the model derived by multiple linear regression was supported by its use of R_{100}. A_8 was a poor predictor of standing biomass for the validation dataset so that whereas inclusion of A_8 strengthened the multiple linear regression model during development it served to increase variance rather than decreasing it. The R_{100} model predicted standing biomass equally well no matter the productivity of the plot.

Because the optical stem density method is based on empirical models and is in its early stages of development, it is desirable to adapt the models as more data become available. In doing so, some of the accuracy can be restored but gains are minimal (Table 6.4). There is a greater difference applying the models to the combined dataset than from modifying the models themselves. This indicates that accuracy via the optical stem density method can be best achieved by increasing the number of plots measured within a plantation rather than tailoring a specific equation. Thus the high degree of precision needed for measuring small plots as part of scientific research ($r^2 > 0.90$ depending on objectives) has not yet been achieved but in a plantation

management scenario accurate results (range of -53 to 57% divergence for single plot) can be gained for the plantation as a whole.

6.II.5 Conclusions

Biomass estimation via optical stem density is a promising method for monitoring biomass accumulation of high density plantations of multi-stemmed willow species grown in SRIC. Initial results indicated high degrees of precision and accuracy. Subsequent validation of the method showed a downgrading of the precision achieved by the method. However, given the speed and ease of the technique, it is possible to sample a large number of plots in a plantation in little time ensuring relatively accurate results (range of -53 to 57% divergence for single plot). The accessibility of instrumentation required for the technique makes biomass estimation via optical stem density a worthwhile method to further develop and enhance for other willow clones and perennial species such as *Populus*.

The R_{100} point of view is by far the most promising. At this time, I recommend the use of R_{100} exclusively for measuring the overall productivity of a plantation and to increase the number of plots measured in order to best account for the uncertainties with the method.

7. SYNTHESIS AND OVERALL CONCLUSIONS

The generalized objective of these studies was to provide direction in how to achieve, maintain and monitor high rates of productivity in willow plantations in Canada. Maintaining high rates of productivity is key to economic feasibility of willow plantations (Keoleian and Volk, 2005). As such, the information gained in these studies will aid in the development of SRIC in Canada specifically the cultivation of willow. By identifying the major factors determining site quality, viable sites for willow production can be identified with realistic expectations. Afforestation is a dramatic change to land use and therefore has consequences for soil nutrient status, soil C storage and soil GHG dynamics. These studies give insight into the effects of willow plantation establishment which aids both in discerning potential issues (e.g. maintaining soil fertility) in advance and allowing for life-cycle analysis and regional planning. This is of critical importance in development of SRIC in Canada as multiple producers and planned adoption is required for the scale required to sustain centralized bioenergy processing facilities. Finally, monitoring productivity non-destructively in an accessible manner simplifies and promotes sound management decisions locally and regionally.

7.1 Findings

Site quality was strongly correlated with the presence of Ca. Calcium, although being an essential element for plant growth, was far more important as a buffer for pH. Not only do soils with a pH of near 8.0 promote availability of nutrients (Havlin et al., 2005), but the presence of Ca was also correlated with abundance of other nutrients as well. Surprisingly, there was little correlation between precipitation or water stress and willow growth at the majority of sites. This indicates that willow does have a future as a prairie crop in Canada, although it should always be kept in mind that acute water stress can severely limit growth as per two sites in this study. Nitrogen and K were found to be the two most nutritionally important elements. However, inorganic N and exchangeable K were both depleted by willow plantations with losses increasing with increased productivity. Therefore these two elements are expected to be the dominant nutritional concerns with maintaining and enhancing productivity in future commercial

operations. Phosphorus demand was found to be low for willow. Also, P tended to be more stable in terms of land conversion than either N or K, though sites with elevated P due to regular fertilization can expect declining P stocks. Increasing exchange capacity both from fine mineral fractions and more soil C led to greater productivity, although heavy clay soils supported less growth than slightly coarser soil as has been noted in previous studies. Soil C stocks generally decreased with land use conversion after the first three year rotation. However, rates of soil respiration indicate that by the second or third year after establishment losses from disturbance and devegetation had stabilized. Greater aboveground biomass was associated with greater soil respiration, indicating that more C is cycling through the soil. Therefore, sites with higher productivity will most likely recover soil C stocks fastest. The development and validation of the optical stem density method for monitoring standing biomass non-destructively will be important in industry development because of the linkages between N and K availability, soil C storage and site productivity. The optical density method of biomass estimation using a 100 cm in row point of view was accurate independent of site on a plantation scale.

7.2 Implications and Recommendations

While total Ca was the best indicator of site quality, it should again be stated that this is more an effect of buffering pH and covariance with nutrient abundance than direct effect of Ca nutrition. Indeed, deficiencies in N and K, as well as isolated incidences of severe water stress, were far more limiting. Therefore, finding calcareous medium to fine textured soils is a good starting point for identifying optimal locations for willow plantations. However, the probability of extreme adverse weather (i.e. drought or extreme cold) should also be considered. Additionally, as deficiencies in N and K are addressed through site selection or amendments it should be expected that other climatic or nutritional factors may become more limiting. It is likely that N and K will require amendments for maximal growth; however, the optimal amount must also take into consideration many other social and environmental factors.

The application of amendments should be made in response to soil nutrient availability and timed to demand (Mitchell et al., 1999). Losses of available nutrients were greatest at high initial concentrations. Also, N and K availability was shown to decrease with increasing

availability. Thus, nutrients are most prone to leaching early in plantation establishment and taken up most rapidly with larger trees. Therefore, application of amendments may be most effective if applied two or three years after conversion. Delaying nutrient amendments will also promote root growth early on (Ericsson, 1981) which will aid the plants in coping with climatic stressors as well as enhancing uptake of nutrients already present in the soil.

The management of plantations will have a tremendous effect on GHG balances. Given that the profitability of bioenergy plantations will depend upon both offsetting use of fossil fuels and below ground C accumulation (Keoleian and Volk, 2005), this is one area that should not be overlooked. As has been demonstrated in traditional agriculture, the use of tillage for site preparation and weed management will cause and maintain soil C deficits (Woods et al., 1992; Zou and Bashkin, 1998; Turner and Lambert, 2000; Six et al., 2002). Additionally, continued tillage between rows will likely minimize the benefits of afforestation on CH_4 fluxes, that is soils will not likely develop the microbial composition of forest soils which have the greatest methane oxidation rates (Le Mer and Roger, 2001).

Harvesting of willow plantations should occur after leaf drop. This allows the maximum amount of nutrients to remain onsite rather than exported with biomass thereby minimizing fertilizer requirements. Additionally, senesced leaves and slash from harvesting are important sources of C.

7.3 Future Research

Willow cultivation as SRIC in Canada is in its infancy and as such there is still much to learn. Of particular value would be long-term studies in which productivity is measured after several rotations to see if it matches results from first rotation. Additionally, manipulative studies where the effects of each nutrient are determined systematically such that the required soil availability be pinpointed for optimal growth. This study identified potential areas of concern for maintaining productivity of plantations into the future. Here again, manipulative long-term studies where yields and soil nutrient stocks in response to varying fertilization regimes need to be evaluated over the full plantation lifecycle.

Monitoring GHG fluxes into and out of the soils on a landscape scale is difficult due to a high degree of spatial and temporal variability. However, continued research is needed to better understand and also quantify the net effects of land use conversion and the willow plantation life-cycle on soil GHGs. In particular, more research should be conducted into how different land-uses react differently to varying climatic conditions. This would aid in explaining the increase in methane oxidation in post-harvest agricultural land not detected in unharvested willow plantations. Although technologically difficult and frequently prohibitively expensive, advances in real time measurements of GHGs would greatly enhance knowledge in the field.

The optical stem density method provided good estimates of standing biomass for the *S. purpurea* 'Hotel' clone. As would be expected, the strength of relationships declined somewhat when validated. However, this loss of precision can be easily overcome when applied to a plantation using multiple sampling points. The need for future research in this area is to apply the technique and equations to other spacings as well as other species to determine its effectiveness and adaptability across a wide range of applications.

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APPENDIX A: SOIL, CLIMATE AND FOLIAR VARIABLES BY SITE FOR DETERMINATION OF SITE QUALITY

Table A.1: Mean exchangeable Ca, Mg, K and Na concentration at nine *S. purpurea* 'Hotel' plantations.

	Exchangeable Cations							
	Ca (SE)		Mg (SE)		K (SE)		Na (SE)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
	$cmol_c\ kg^{-1}$							
ELL	38.2 (3.4)	33.9 (2.3)	0.05 (0.04)	0.55 (0.18)	0.66 (0.11)	0.32 (0.03)	0.00 (0.00)	0.01 (0.01)
SMO	4.1 (0.7)	3.7 (0.7)	0.00 (0.00)	0.00 (0.00)	0.22 (0.05)	0.14 (0.02)	0.02 (0.02)	0.02 (0.02)
LAK	23.9 (1.0)	18.8 (1.3)	0.00 (0.00)	0.00 (0.00)	1.33 (0.13)	0.42 (0.05)	0.01 (0.01)	0.01 (0.01)
UOS	14.7 (1.3)	12.8 (1.2)	12.63 (1.65)	18.01 (1.47)	1.24 (0.11)	0.78 (0.05)	1.06 (0.26)	3.60 (0.74)
POR	29.2 (1.5)	23.7 (3.1)	0.15 (0.14)	0.24 (0.20)	0.93 (0.05)	0.39 (0.03)	0.00 (0.00)	0.02 (0.01)
BIR	34.3 (1.0)	22.1 (2.0)	17.93 (2.13)	21.35 (2.66)	0.96 (0.08)	0.66 (0.05)	0.03 (0.02)	0.10 (0.07)
SSM	1.7 (0.1)	1.2 (0.1)	0.24 (0.03)	0.12 (0.02)	0.09 (0.00)	0.07 (0.00)	0.00 (0.00)	0.00 (0.00)
GUE	14.6 (0.5)	13.7 (0.4)	2.47 (0.30)	2.00 (0.19)	0.32 (0.03)	0.16 (0.01)	0.03 (0.02)	0.03 (0.01)
PIC	22.4 (1.7)	19.0 (1.5)	1.52 (0.21)	1.31 (0.23)	0.44 (0.04)	0.28 (0.03)	0.00 (0.00)	0.00 (0.00)
All Sites	20.33 (4.2)	16.56 (3.4)	3.888 (2.22)	4.841 (2.83)	0.687 (0.15)	0.357 (0.08)	0.128 (0.12)	0.420 (0.40)

Table A.2: Exchangeable Al and Fe concentration and Cation Exchange Capacity for nine *S. purpurea* 'Hotel' plantations.

	Exchangeable Cations				Cation Exchange Capacity (CEC) (SE)	
	Al (SE)		Fe (SE)			
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
	$cmol_c\ kg^{-1}$					
ELL	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	38.9 (3.4)	34.8 (2.5)
SMO	0.06 (0.02)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	4.4 (0.7)	3.8 (0.7)
LAK	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	25.2 (1.0)	19.3 (1.3)
UOS	0.00 (0.00)	0.00 (0.00)	0.04 (0.01)	0.05 (0.01)	29.7 (2.5)	35.3 (2.1)
POR	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	30.2 (1.6)	24.4 (3.2)
BIR	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	53.2 (1.5)	44.2 (2.5)
SSM	0.10 (0.01)	0.09 (0.01)	0.01 (0.00)	0.01 (0.00)	2.2 (0.1)	1.5 (0.1)
GUE	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	17.5 (0.7)	15.9 (0.3)
PIC	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.3 (1.8)	20.6 (1.7)
All Sites	0.020 (0.01)	0.013 (0.01)	0.007 (0.00)	0.007 (0.00)	25.06 (5.3)	22.20 (4.8)

Table A.3: Mean soil inorganic and total N concentration at nine *S. purpurea* 'Hotel' plantations.

	NH_4^+ (SE)		NO_3^- (SE)		Inorganic N (SE)		Total N (SE)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
	—— μg N g^{-1} ——						—— mg g^{-1} ——	
ELL	8.97 (0.41)	6.61 (0.62)	4.20 (0.41)	2.01 (0.77)	13.2 (0.8)	8.6 (1.3)	N/A†	N/A
SMO	4.28 (0.98)	1.65 (0.27)	1.82 (0.68)	0.20 (0.13)	6.1 (1.4)	1.8 (0.3)	1.82 (0.17)	0.71 (0.15)
LAK	0.74 (0.18)	0.70 (0.26)	0.84 (0.30)	0.15 (0.08)	1.6 (0.4)	0.9 (0.3)	2.37 (0.10)	1.31 (0.16)
UOS	13.86 (1.16)	13.54 (1.39)	0.22 (0.10)	0.08 (0.06)	14.1 (1.2)	13.6 (1.4)	2.36 (0.15)	1.96 (0.12)
POR	6.30 (0.47)	5.79 (0.33)	0.58 (0.13)	0.33 (0.20)	6.9 (0.5)	6.1 (0.5)	3.03 (0.17)	2.13 (0.22)
BIR	7.31 (0.71)	4.75 (1.03)	1.15 (0.16)	0.28 (0.10)	8.5 (0.8)	5.0 (1.1)	N/A	N/A
SSM	10.39 (2.40)	5.52 (0.92)	1.05 (0.28)	0.56 (0.28)	11.4 (2.3)	6.1 (0.8)	1.75 (0.05)	1.34 (0.16)
GUE	8.33 (1.13)	7.95 (0.77)	1.61 (0.41)	0.69 (0.11)	9.9 (1.4)	8.6 (0.9)	1.70 (0.08)	1.61 (0.10)
PIC	7.81 (0.64)	5.23 (0.97)	1.14 (0.28)	0.44 (0.22)	8.9 (0.9)	5.7 (1.2)	1.98 (0.16)	1.18 (0.18)
All Sites	7.554 (1.23)	5.750 (1.24)	1.400 (0.39)	0.525 (0.20)	8.95 (1.3)	6.28 (1.3)	2.14 (0.18)	1.46 (0.18)

†N/A = results not available

Table A.4: Mean soil C:N ratio total and organic C and leachable P concentration at nine *S. purpurea* 'Hotel' plantations.

	C:N Ratio (SE)		Total C (SE)		Total Organic C (SE)		Leachable P (SE)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
			mg g^{-1}					
ELL	N/A†	N/A	68.8 (3.5)	48.2 (1.8)	N/A	N/A	1.04 (0.03)	0.85 (0.02)
SMO	10.2 (0.36)	6.9 (1.54)	21.2 (2.3)	7.0 (2.2)	18.59 (2.01)	5.71 (2.28)	0.62 (0.09)	0.44 (0.03)
LAK	13.2 (0.35)	19.6 (1.68)	35.0 (0.5)	27.6 (1.2)	31.11 (0.74)	24.62 (1.20)	0.63 (0.02)	0.61 (0.04)
UOS	10.2 (0.81)	9.9 (0.92)	24.2 (0.3)	20.2 (1.5)	23.64 (0.36)	19.09 (1.50)	0.67 (0.03)	0.68 (0.01)
POR	12.5 (0.32)	15.5 (0.68)	38.4 (2.3)	37.4 (1.8)	37.71 (1.23)	32.41 (1.84)	0.98 (0.02)	0.81 (0.01)
BIR	N/A	N/A	42.1 (1.3)	30.1 (2.6)	N/A	N/A	0.66 (0.02)	0.55 (0.02)
SSM	13.9 (0.50)	11.1 (0.88)	22.7 (0.6)	14.3 (1.1)	24.35 (0.52)	14.50 (1.15)	0.35 (0.03)	0.33 (0.02)
GUE	13.9 (0.43)	12.1 (0.42)	24.3 (1.3)	20.9 (1.0)	23.61 (1.22)	19.30 (0.82)	1.03 (0.03)	1.01 (0.07)
PIC	10.8 (0.43)	23.2 (9.82)	22.2 (1.2)	22.2 (3.8)	21.12 (1.20)	20.87 (3.76)	1.15 (0.03)	0.98 (0.04)
All Sites	12.1 (0.64)	14.0 (2.17)	33.21 (5.2)	25.33 (4.1)	25.73 (2.47)	19.50 (3.12)	0.792 (0.09)	0.695 (0.08)

†N/A = results not available

Table A.5: Soil pH and texture at nine *S. purpurea* 'Hotel' plantations.

	pH (SE)		Sand (SE)		Silt (SE)		Clay (SE)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
			g g^{-1}					
ELL	6.04 (0.11)	6.76 (0.17)	0.15 (0.01)	0.14 (0.01)	0.48 (0.01)	0.48 (0.01)	0.37 (0.01)	0.38 (0.01)
SMO	5.08 (0.14)	5.77 (0.13)	0.74 (0.04)	0.80 (0.01)	0.14 (0.02)	0.13 (0.01)	0.12 (0.02)	0.08 (0.01)
LAK	8.26 (0.03)	8.40 (0.02)	0.14 (0.03)	0.50 (0.03)	0.48 (0.03)	0.39 (0.03)	0.37 (0.02)	0.11 (0.01)
UOS	6.60 (0.12)	7.43 (0.25)	0.18 (0.03)	0.12 (0.02)	0.34 (0.04)	0.32 (0.08)	0.48 (0.04)	0.56 (0.07)
POR	8.27 (0.02)	8.38 (0.03)	0.12 (0.02)	0.39 (0.03)	0.63 (0.04)	0.51 (0.03)	0.25 (0.05)	0.10 (0.00)
BIR	8.27 (0.05)	8.63 (0.08)	0.03 (0.00)	0.03 (0.00)	0.27 (0.01)	0.26 (0.02)	0.70 (0.00)	0.72 (0.02)
SSM	5.44 (0.05)	5.52 (0.10)	0.56 (0.03)	0.58 (0.05)	0.25 (0.03)	0.28 (0.04)	0.20 (0.04)	0.14 (0.03)
GUE	6.89 (0.05)	6.83 (0.04)	0.43 (0.03)	0.41 (0.03)	0.32 (0.02)	0.40 (0.04)	0.24 (0.03)	0.19 (0.02)
PIC	6.81 (0.16)	6.94 (0.17)	0.27 (0.03)	0.29 (0.04)	0.41 (0.02)	0.44 (0.06)	0.33 (0.04)	0.27 (0.06)
All Sites	6.849 (0.41)	7.185 (0.38)	0.291 (0.08)	0.361 (0.08)	0.369 (0.05)	0.356 (0.04)	0.340 (0.06)	0.283 (0.08)

Table A.6: Modeled precipitation and water stress as indicated by $\delta^{13}C$ at nine *S. purpurea* 'Hotel' plantations.

	Mean Annual Precipitation (SE)†		Summer Precipitation (SE)†		
	30 Year Mean	Production Years	Years 1-3	Years 1-2	$\delta^{13}C$
	mm				
SMO	432 (13)	412 (27)	276 (15)	288 (17)	-26.12 (0.18)
LAK	349 (16)	454 (53)	326 (41)	366 (19)	-27.37 (0.29)
UOS	364 (17)	382 (74)	265 (57)	315 (46)	-25.01 (0.12)
POR	537 (17)	523 (88)	318 (84)	342 (139)	-27.77 (0.21)
SSM	914 (26)	826 (56)	332 (11)	325 (14)	-29.32 (0.10)
GUE	894 (26)	971 (143)	418 (90)	357 (114)	-28.08 (0.10)
PIC	887 (18)	948 (131)	404 (102)	328 (117)	-28.48 (0.20)
All Sites	625 (99)	645 (98)	334 (22)	331 (10)	-27.45 (0.55)

†Modeled with BioSIM.

Table A.7: Modeled degree days, vapour pressure, potential evapotranspiration (PET), total radiation and aridity index at nine *S. purpurea* 'Hotel' plantations.

	Degree Days (SE)†	Vapour Pressure (SE)†	PET (SE)†	Total Radiation (SE)†	Aridity Index (SE)†
	°C day	kPa	mm	MJ m^{-1}	cm
SMO	1,330	184	511	3,902	12.4
LAK	1,822	226	570	4,008	16.1
UOS	1,792	231	561	4,088	17.9
POR	1,890	171	575	4,175	17.1
SSM	1,729	193	554	4,442	10.7
GUE	2,058	187	568	4,488	10.1
PIC	2,300	225	603	4,401	12.0
All Sites	1,845 (113)	202 (9)	563 (10)	4,215 (87)	13.8 (1.2)

†Modeled with BioSIM.

Table A.8: Foliar Ca, Mg, K and Na concentration at nine *S. purpurea* 'Hotel' plantations.

	Ca (SE)		Mg (SE)		K (SE)		Na (SE)	
	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem
	mg g^{-1}							
ELL	14.9 (0.7)	N/A†	3.4 (0.2)	N/A	11.4 (0.4)	N/A	0.1 (0.0)	N/A
SMO	8.4 (2.1)	5.0 (0.4)	2.1 (0.6)	0.7 (0.0)	10.1 (2.6)	2.8 (0.1)	0.0 (0.0)	0.0 (0.0)
LAK	14.8 (0.6)	4.9 (0.4)	3.1 (0.2)	0.8 (0.1)	13.2 (0.4)	1.9 (0.1)	0.0 (0.0)	0.0 (0.0)
UOS	11.0 (0.5)	4.1 (0.3)	5.8 (0.4)	1.3 (0.1)	11.0 (0.6)	3.2 (0.2)	0.0 (0.0)	0.1 (0.0)
POR	15.9 (0.6)	5.2 (0.3)	3.2 (0.1)	0.7 (0.0)	14.3 (0.5)	1.8 (0.1)	0.0 (0.0)	0.0 (0.0)
BIR	11.5 (0.6)	N/A	5.8 (0.2)	N/A	10.0 (0.3)	N/A	0.0 (0.0)	N/A
SSM	15.2 (1.3)	5.8 (0.5)	4.0 (0.5)	0.7 (0.0)	5.3 (0.4)	1.5 (0.2)	0.0 (0.0)	0.0 (0.0)
GUE	19.0 (0.6)	5.9 (0.4)	3.9 (0.1)	0.6 (0.0)	8.5 (0.7)	1.6 (0.1)	0.1 (0.0)	0.0 (0.0)
PIC	18.9 (1.3)	5.2 (0.3)	2.3 (0.1)	0.4 (0.0)	12.7 (0.7)	2.1 (0.0)	0.1 (0.0)	0.0 (0.0)
All Sites	14.4 (1.2)	5.2 (0.2)	3.7 (0.4)	0.7 (0.1)	10.7 (0.9)	2.1 (0.2)	0.0 (0.0)	0.0 (0.0)

†N/A = results not available.

Table A.9: Foliar N, P and C concentration at nine *S. purpurea* 'Hotel' plantations.

	N (SE)		P (SE)		C (SE)	
	Leaves	Stem	Leaves	Stem	Leaves	Stem
	mg g^{-1}				g g^{-1}	
ELL	32.5 (1.7)	N/A†	2.4 (0.1)	N/A	0.48 (0.00)	N/A
SMO	25.9 (6.8)	7.3 (0.5)	2.0 (0.5)	1.0 (0.1)	0.39 (0.10)	0.48 (0.00)
LAK	30.3 (1.6)	6.8 (0.3)	1.8 (0.1)	0.7 (0.1)	0.48 (0.00)	0.48 (0.00)
UOS	28.5 (1.5)	7.1 (0.4)	2.2 (0.1)	1.0 (0.0)	0.49 (0.00)	0.49 (0.00)
POR	26.3 (3.0)	4.5 (0.4)	2.0 (0.1)	0.7 (0.0)	0.48 (0.00)	0.48 (0.00)
BIR	25.3 (1.3)	N/A	1.6 (0.1)	N/A	0.48 (0.00)	N/A
SSM	20.2 (0.5)	6.8 (0.5)	1.5 (0.2)	0.6 (0.1)	0.48 (0.00)	0.49 (0.00)
GUE	21.7 (1.6)	5.4 (0.4)	2.4 (0.1)	1.0 (0.1)	0.47 (0.00)	0.47 (0.01)
PIC	26.3 (1.6)	7.1 (0.4)	3.2 (0.5)	1.1 (0.1)	0.48 (0.00)	0.50 (0.02)
All Sites	26.3 (1.3)	6.4 (0.4)	2.1 (0.2)	0.9 (0.1)	0.47 (0.01)	0.49 (0.00)

†N/A = results not available.

Table A.10: Multiple linear regression models for predicting productivity from soil and climatic variables.

Method	Model / Variable	Adj. r^2	*P*	SEE†	SS‡
Stepwise	y = 5.66 - 1.72 Total Fe + 4.61Total Ca	0.825	.000	3.13	1587
	Total Fe		.000		158
	Total Ca		.000		1429
Stepwise	y = 6.21 - 0.641 Total Al + 4.55 Total Ca	0.817	.000	3.19	1574
	Total Al		.000		163
	Total Ca		.000		1411
Stepwise	y = 8.68 + 11.51 Clay (20-40 cm) - 1.34 Total Al + 5.41 Total Ca	0.839	.000	3.00	1623
	Clay (20-40 cm)		.000		153
	Total Al		.000		499
	Total Ca		.000		971
Stepwise	y = 31.46 - 3.00 pH (20-40 cm) - 0.0455 sum. precip. + 6.30 Total Ca	0.839	.000	3.00	1622
	pH (20-40 cm)		.000		906
	sum. precip.		.002		106
	Total Ca		.000		610
Forward	y = 6.09 + 6.98 Clay (20-40 cm) - 2.60 Total Fe + 5.09 Total Ca	0.834	.000	3.05	1613
	Clay (20-40 cm)		.000		153
	Total Fe		.000		371
	Total Ca		.000		1089
Forward	y = 12.24 - 0.490 C:N (0-20 cm) -2.11 Total Fe + 5.00 Total Ca	0.832	.000	3.06	1610
	C:N (0-20 cm)		.000		260
	Total Fe		.000		191
	Total Ca		.000		1159

†SEE = Standard error of estimate.
‡SS = Sum of squares.

APPENDIX B: DIFFERENCES IN SOIL PROPERTIES BETWEEN NINE *SALIX* PLANTATIONS AND REFERENCE SITES ACROSS CANADA

Table B.1: Exchangeable Ca, Mg, K and Na concentration for plantations and reference sites.

	Exchangeable Cations							
	Ca		Mg		K		Na	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
	$cmol_c\ kg^{-1}$							
ELL								
Salix Mean (SE)	38.2 (3.4)	33.9 (2.3)	0.05 (0.04)	0.55 (0.18)	0.66 (0.11)	0.32 (0.03)	0.00 (0.00)	0.01 (0.01)
Ref. Mean (SE)	40.1 (3.5)	31.6 (1.6)	0.31 (0.10)	0.38 (0.23)	0.82 (0.10)	0.30 (0.02)	0.02 (0.01)	0.04 (0.00)
Difference	-1.9	2.3	-0.26*	0.17	-0.16†	0.02†	-0.02**	-0.03**
SMO								
Salix Mean (SE)	4.1 (0.7)	3.7 (0.7)	0.00 (0.00)	0.00 (0.00)	0.22 (0.05)	0.14 (0.02)	0.02 (0.02)	0.02 (0.02)
Ref. Mean (SE)	6.0 (1.2)	4.1 (1.3)	0.00 (0.00)	0.00 (0.00)	0.25 (0.04)	0.21 (0.04)	0.14 (0.04)	0.10 (0.03)
Difference	-2.0	-0.5	0.00	0.00	-0.03†	-0.07†	-0.12*	-0.09*
LAK								
Salix Mean (SE)	23.9 (1.0)	18.8 (1.3)	0.00 (0.00)	0.00 (0.00)	1.33 (0.13)	0.42 (0.05)	0.01 (0.01)	0.01 (0.01)
Ref. Mean (SE)	31.7 (0.3)	24.7 (2.7)	0.46 (0.19)	1.19 (0.72)	1.45 (0.06)	0.68 (0.11)	0.02 (0.01)	0.03 (0.02)
Difference	-7.9***	-5.9	-0.46*	-1.19	-0.12†	-0.27†	-0.01†	-0.01†
UOS								
Salix Mean (SE)	14.7 (1.3)	12.8 (1.2)	12.63 (1.65)	18.01 (1.47)	1.24 (0.11)	0.78 (0.05)	1.06 (0.26)	3.60 (0.74)
Ref. Mean (SE)	18.3 (2.3)	16.7 (1.2)	13.19 (0.77)	20.50 (1.41)	0.94 (0.07)	0.73 (0.01)	1.25 (0.20)	3.07 (0.43)
Difference	-3.5	-3.9	-0.57	-2.50	0.30†	0.05†	-0.20†	0.53†
POR								
Salix Mean (SE)	29.2 (1.5)	23.7 (3.1)	0.15 (0.14)	0.24 (0.20)	0.93 (0.05)	0.39 (0.03)	0.00 (0.00)	0.02 (0.01)
Ref. Mean (SE)	27.8 (0.2)	28.0 (1.4)	0.00 (0.00)	0.00 (0.00)	1.37 (0.06)	0.75 (0.06)	0.00 (0.00)	0.00 (0.00)
Difference	1.4	-4.2	0.15	0.24	-0.44***	-0.36***	0.00	0.02*
BIR								
Salix Mean (SE)	34.3 (1.0)	22.1 (2.0)	17.93 (2.13)	21.35 (2.66)	0.96 (0.08)	0.66 (0.05)	0.03 (0.02)	0.10 (0.07)
Ref. Mean (SE)	35.7 (3.2)	29.0 (3.3)	14.58 (1.40)	18.75 (1.82)	1.55 (0.14)	0.84 (0.14)	0.05 (0.02)	0.07 (0.03)
Difference	-1.4	-7.0	3.35	2.61	-0.59**	-0.17†	-0.02†	0.03†
SSM								
Salix Mean (SE)	1.7 (0.1)	1.2 (0.1)	0.24 (0.03)	0.12 (0.02)	0.09 (0.00)	0.07 (0.00)	0.00 (0.00)	0.00 (0.00)
Ref. Mean (SE)	1.7 (0.2)	0.7 (0.1)	0.20 (0.03)	0.04 (0.01)	0.09 (0.00)	0.06 (0.00)	0.00 (0.00)	0.00 (0.00)
Difference	0.1	0.5*	0.05	0.08*	0.00†	0.01*	0.00	0.00
GUE								
Salix Mean (SE)	14.6 (0.5)	13.7 (0.4)	2.47 (0.30)	2.00 (0.19)	0.32 (0.03)	0.16 (0.01)	0.03 (0.02)	0.03 (0.01)
Ref. Mean (SE)	15.5 (0.3)	14.2 (0.3)	1.85 (0.11)	1.66 (0.11)	0.25 (0.01)	0.16 (0.01)	0.08 (0.01)	0.09 (0.00)
Difference	-0.9	-0.5	0.61	0.34	0.07†	-0.00†	-0.05*	-0.06**
PIC								
Salix Mean (SE)	22.4 (1.7)	19.0 (1.5)	1.52 (0.21)	1.31 (0.23)	0.44 (0.04)	0.28 (0.03)	0.00 (0.00)	0.00 (0.00)
Ref. Mean (SE)	25.1 (0.6)	23.0 (1.1)	1.74 (0.14)	2.03 (0.25)	0.47 (0.02)	0.37 (0.01)	0.00 (0.00)	0.00 (0.00)
Difference	-2.7	-4.0	-0.22	-0.72	-0.03†	-0.09*	0.00	0.00
All Sites								
Salix Mean (SE)	20.33 (4.2)	16.56 (3.4)	3.888 (2.22)	4.841 (2.83)	0.687 (0.15)	0.357 (0.08)	0.128 (0.12)	0.420 (0.40)
Ref. Mean (SE)	22.43 (4.4)	19.13 (3.7)	3.593 (1.96)	4.950 (2.79)	0.798 (0.19)	0.456 (0.10)	0.174 (0.14)	0.377 (0.34)
Difference	-2.10*	-2.57*	0.295	-0.109	-0.112†	-0.098†	-0.046†	0.044†
Slope	0.95	0.88	1.116	1.001	0.707†	0.711†	0.853**	1.180***
Intercept	-0.89*	-0.27*	-0.124	-0.112	0.122†	0.033†	-0.021†	-0.024†

†Significant at $P < 0.10$.
*Significant at $P < 0.05$.
**Significant at $P < 0.01$.
***Significant at $P < 0.001$.

Table B.2: Exchangeable Al, Fe concentration and Cation Exchange Capacity (CEC) all plantations and reference sites.

	Exchangeable Cations				Cation Exchange Capacity (CEC)	
	Al		Fe			
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
	$cmol_c\ kg^{-1}$					
ELL						
Salix Mean (SE)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	38.9 (3.4)	34.8 (2.5)
Ref. Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	41.3 (3.5)	32.3 (1.7)
Difference	0.00	0.00†	0.00†	-0.00†	-2.4	2.4
SMO						
Salix Mean (SE)	0.06 (0.02)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	4.4 (0.7)	3.8 (0.7)
Ref. Mean (SE)	0.02 (0.01)	0.02 (0.00)	0.00 (0.00)	0.00 (0.00)	6.4 (1.3)	4.5 (1.3)
Difference	0.05*	-0.00†	0.00†	0.00†	-2.1	-0.6
LAK						
Salix Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	25.2 (1.0)	19.3 (1.3)
Ref. Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	33.7 (0.4)	26.6 (3.3)
Difference	0.00	0.00†	-0.00†	0.00†	-8.5***	-7.3
UOS						
Salix Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.04 (0.01)	0.05 (0.01)	29.7 (2.5)	35.3 (2.1)
Ref. Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.07 (0.01)	0.07 (0.01)	33.7 (2.6)	41.1 (1.0)
Difference	-0.00**	-0.00*	-0.02†	-0.02†	-4.0	-5.8*
POR						
Salix Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	30.2 (1.6)	24.4 (3.2)
Ref. Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	29.1 (0.3)	28.7 (1.4)
Difference	-0.00	-0.00†	0.00†	-0.00†	1.1	-4.3
BIR						
Salix Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	53.2 (1.5)	44.2 (2.5)
Ref. Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	51.9 (2.1)	48.7 (3.9)
Difference	-0.00	0.00†	-0.00†	-0.00†	1.3	-4.5
SSM						
Salix Mean (SE)	0.10 (0.01)	0.09 (0.01)	0.01 (0.00)	0.01 (0.00)	2.2 (0.1)	1.5 (0.1)
Ref. Mean (SE)	0.11 (0.01)	0.11 (0.01)	0.01 (0.00)	0.01 (0.00)	2.1 (0.2)	0.9 (0.1)
Difference	-0.02	-0.02†	0.00†	-0.00†	0.1	0.6**
GUE						
Salix Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	17.5 (0.7)	15.9 (0.3)
Ref. Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	17.7 (0.4)	16.1 (0.4)
Difference	0.00	0.00†	0.00	0.00†	-0.3	-0.2
PIC						
Salix Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.3 (1.8)	20.6 (1.7)
Ref. Mean (SE)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	27.3 (0.6)	25.4 (1.1)
Difference	-0.00	-0.00†	0.00	0.00	-3.0	-4.8*
All Sites						
Salix Mean (SE)	0.020 (0.01)	0.013 (0.01)	0.007 (0.00)	0.007 (0.00)	25.06 (5.3)	22.20 (4.8)
Ref. Mean (SE)	0.017 (0.01)	0.015 (0.01)	0.010 (0.01)	0.010 (0.01)	27.03 (5.3)	24.94 (5.2)
Difference	0.003	-0.002†	-0.003†	-0.002†	-1.97	-2.74*
Slope	0.837	0.800***	0.643***	0.674***	0.98	0.89
Intercept	0.006	0.001†	0.001†	0.001†	-1.44	-0.04*

†Significant at $P < 0.10$.
*Significant at $P < 0.05$.
**Significant at $P < 0.01$.
***Significant at $P < 0.001$.

Table B.3: Inorganic N concentration for nine *Salix* plantations and reference sites.

	NH_4^+		NO_3		Inorganic N	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
	μg N g^{-1}					
ELL						
Salix Mean (SE)	8.97 (0.41)	6.61 (0.62)	4.20 (0.41)	2.01 (0.77)	13.2 (0.8)	8.6 (1.3)
Reference Mean (SE)	8.37 (0.47)	4.83 (0.44)	2.79 (0.47)	0.48 (0.16)	11.2 (0.6)	5.3 (0.4)
Difference	0.60†	1.78*	1.41†	1.53†	2.0†	3.3*
SMO						
Salix Mean (SE)	4.28 (0.98)	1.65 (0.27)	1.82 (0.68)	0.20 (0.13)	6.1 (1.4)	1.8 (0.3)
Reference Mean (SE)	2.48 (1.14)	1.66 (1.03)	0.14 (0.14)	0.00 (0.00)	2.6 (1.1)	1.7 (1.0)
Difference	1.80†	-0.01†	1.68*	0.20†	3.5†	0.2†
LAK						
Salix Mean (SE)	0.74 (0.18)	0.70 (0.26)	0.84 (0.30)	0.15 (0.08)	1.6 (0.4)	0.9 (0.3)
Reference Mean (SE)	1.58 (0.16)	3.14 (0.92)	6.84 (2.21)	1.03 (0.28)	8.4 (2.3)	4.2 (1.1)
Difference	-0.84**	-2.44*	-6.00*	-0.88*	-6.8*	-3.3*
UOS						
Salix Mean (SE)	13.86 (1.16)	13.54 (1.39)	0.22 (0.10)	0.08 (0.06)	14.1 (1.2)	13.6 (1.4)
Reference Mean (SE)	16.05 (2.03)	15.10 (1.38)	1.58 (0.32)	0.08 (0.04)	17.6 (2.0)	15.2 (1.4)
Difference	-2.19†	-1.56†	-1.37**	0.00†	-3.6†	-1.6†
POR						
Salix Mean (SE)	6.30 (0.47)	5.79 (0.33)	0.58 (0.13)	0.33 (0.20)	6.9 (0.5)	6.1 (0.5)
Reference Mean (SE)	8.14 (1.30)	9.18 (0.91)	13.85 (0.47)	10.68 (0.97)	22.0 (1.7)	19.9 (1.4)
Difference	-1.84†	-3.39**	-13.27***	-10.34***	-15.1***	-13.7***
BIR						
Salix Mean (SE)	7.31 (0.71)	4.75 (1.03)	1.15 (0.16)	0.28 (0.10)	8.5 (0.8)	5.0 (1.1)
Reference Mean (SE)	7.04 (0.40)	4.70 (0.54)	4.13 (3.44)	2.51 (2.33)	11.2 (3.4)	7.2 (2.7)
Difference	0.28†	0.06†	-2.98†	-2.23†	-2.7†	-2.2†
SSM						
Salix Mean (SE)	10.39 (2.40)	5.52 (0.92)	1.05 (0.28)	0.56 (0.28)	11.4 (2.3)	6.1 (0.8)
Reference Mean (SE)	11.47 (0.82)	4.40 (1.01)	0.09 (0.06)	0.04 (0.04)	11.6 (0.8)	4.4 (1.0)
Difference	-1.08†	1.12†	0.96**	0.52†	-0.1†	1.6†
GUE						
Salix Mean (SE)	8.33 (1.13)	7.95 (0.77)	1.61 (0.41)	0.69 (0.11)	9.9 (1.4)	8.6 (0.9)
Reference Mean (SE)	6.87 (1.57)	5.81 (1.12)	1.87 (0.64)	0.69 (0.20)	8.7 (1.5)	6.5 (1.3)
Difference	1.47†	2.14†	-0.26†	0.00†	1.2†	2.1†
PIC						
Salix Mean (SE)	7.81 (0.64)	5.23 (0.97)	1.14 (0.28)	0.44 (0.22)	8.9 (0.9)	5.7 (1.2)
Reference Mean (SE)	14.54 (2.29)	15.35 (2.61)	0.86 (0.14)	0.22 (0.09)	15.4 (2.3)	15.6 (2.6)
Difference	-6.74*	-10.12**	0.28†	0.22†	-6.5*	-9.9**
All Sites						
Salix Mean (SE)	7.554 (1.23)	5.750 (1.24)	1.400 (0.39)	0.525 (0.20)	8.95 (1.3)	6.28 (1.3)
Reference Mean (SE)	8.504 (1.63)	7.130 (1.67)	3.572 (1.47)	1.747 (1.15)	12.08 (1.9)	8.88 (2.1)
Difference	-0.949†	-1.380†	-2.172†	-1.222†	-3.12†	-2.60†
Slope	0.646*	0.490*	-0.065***	-0.024***	0.21*	0.27**
Intercept	2.057†	2.260†	1.632†	0.567†	6.41†	3.86†

†Significant at $P < 0.10$.
*Significant at $P < 0.05$.
**Significant at $P < 0.01$.
***Significant at $P < 0.001$.

Table B.4: Total C, leachable P concentration and pH for nine *Salix* plantations and reference sites.

	Total C		Leachable P		pH	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
	mg g^{-1}					
ELL						
Salix Mean (SE)	68.8 (3.5)	48.2 (1.8)	1.04 (0.03)	0.85 (0.02)	6.04 (0.11)	6.76 (0.17)
Reference Mean (SE)	70.7 (3.0)	38.5 (2.6)	1.17 (0.03)	0.81 (0.04)	6.38 (0.05)	6.76 (0.25)
Difference	-1.9	9.7*	-0.13*	0.04†	-0.34*	-0.00†
SMO						
Salix Mean (SE)	21.2 (2.3)	7.0 (2.2)	0.62 (0.09)	0.44 (0.03)	5.08 (0.14)	5.77 (0.13)
Reference Mean (SE)	22.0 (3.1)	7.9 (1.0)	0.33 (0.04)	0.33 (0.05)	5.70 (0.11)	5.77 (0.16)
Difference	-0.8	-0.9	0.28*	0.12†	-0.62**	-0.01†
LAK						
Salix Mean (SE)	35.0 (0.5)	27.6 (1.2)	0.63 (0.02)	0.61 (0.04)	8.26 (0.03)	8.40 (0.02)
Reference Mean (SE)	35.3 (1.1)	30.6 (2.6)	0.62 (0.02)	0.57 (0.02)	8.21 (0.05)	8.35 (0.04)
Difference	-0.4	-3.0	0.01†	0.04†	0.05	0.05†
UOS						
Salix Mean (SE)	24.2 (0.3)	20.2 (1.5)	0.67 (0.03)	0.68 (0.01)	6.60 (0.12)	7.43 (0.25)
Reference Mean (SE)	28.0 (1.3)	23.1 (1.1)	0.77 (0.02)	0.62 (0.01)	6.46 (0.09)	7.12 (0.08)
Difference	-3.8*	-2.8	-0.10*	0.06*	0.14	0.31†
POR						
Salix Mean (SE)	38.4 (2.3)	37.4 (1.8)	0.98 (0.02)	0.81 (0.01)	8.27 (0.02)	8.38 (0.03)
Reference Mean (SE)	37.0 (0.6)	35.7 (1.4)	0.98 (0.02)	0.87 (0.02)	8.12 (0.01)	8.19 (0.04)
Difference	1.4	1.8	-0.00†	-0.07*	0.15***	0.19**
BIR						
Salix Mean (SE)	42.1 (1.3)	30.1 (2.6)	0.66 (0.02)	0.55 (0.02)	8.27 (0.05)	8.63 (0.08)
Reference Mean (SE)	48.1 (2.8)	39.7 (3.7)	0.83 (0.02)	0.68 (0.07)	8.13 (0.07)	8.24 (0.18)
Difference	-6.0	-9.6	-0.16***	-0.13†	0.14	0.39†
SSM						
Salix Mean (SE)	22.7 (0.6)	14.3 (1.1)	0.35 (0.03)	0.33 (0.02)	5.44 (0.05)	5.52 (0.10)
Reference Mean (SE)	27.2 (1.9)	13.9 (1.1)	0.36 (0.03)	0.32 (0.02)	5.41 (0.07)	5.60 (0.10)
Difference	-4.5	0.4	-0.01†	0.01†	0.02	-0.08†
GUE						
Salix Mean (SE)	24.3 (1.3)	20.9 (1.0)	1.03 (0.03)	1.01 (0.07)	6.89 (0.05)	6.83 (0.04)
Reference Mean (SE)	29.0 (0.9)	25.4 (3.2)	1.18 (0.03)	1.08 (0.04)	6.99 (0.07)	6.91 (0.10)
Difference	-4.7*	-4.5	-0.15**	-0.08†	-0.10	-0.08†
PIC						
Salix Mean (SE)	22.2 (1.2)	22.2 (3.8)	1.15 (0.03)	0.98 (0.04)	6.81 (0.16)	6.94 (0.17)
Reference Mean (SE)	21.5 (1.7)	17.1 (2.0)	1.16 (0.02)	1.14 (0.04)	7.09 (0.06)	7.04 (0.05)
Difference	0.7	5.1	-0.01†	-0.16*	-0.28	-0.10†
All Sites						
Salix Mean (SE)	33.21 (5.2)	25.33 (4.1)	0.792 (0.09)	0.695 (0.08)	6.849 (0.41)	7.185 (0.38)
Reference Mean (SE)	35.43 (5.2)	25.77 (3.8)	0.824 (0.11)	0.713 (0.10)	6.942 (0.35)	7.109 (0.34)
Difference	-2.22*	-0.43	-0.031†	-0.018†	-0.093	0.076†
Slope	0.98	0.97	0.737†	0.769*	1.137	1.107†
Intercept	-1.40*	0.34	0.185†	0.147†	-1.043	-0.687†

†Significant at $P < 0.10$; *Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$.

APPENDIX C: CUMULATIVE N_2O EMISSIONS AT UOS (2007-2009) AND LAK (2007)

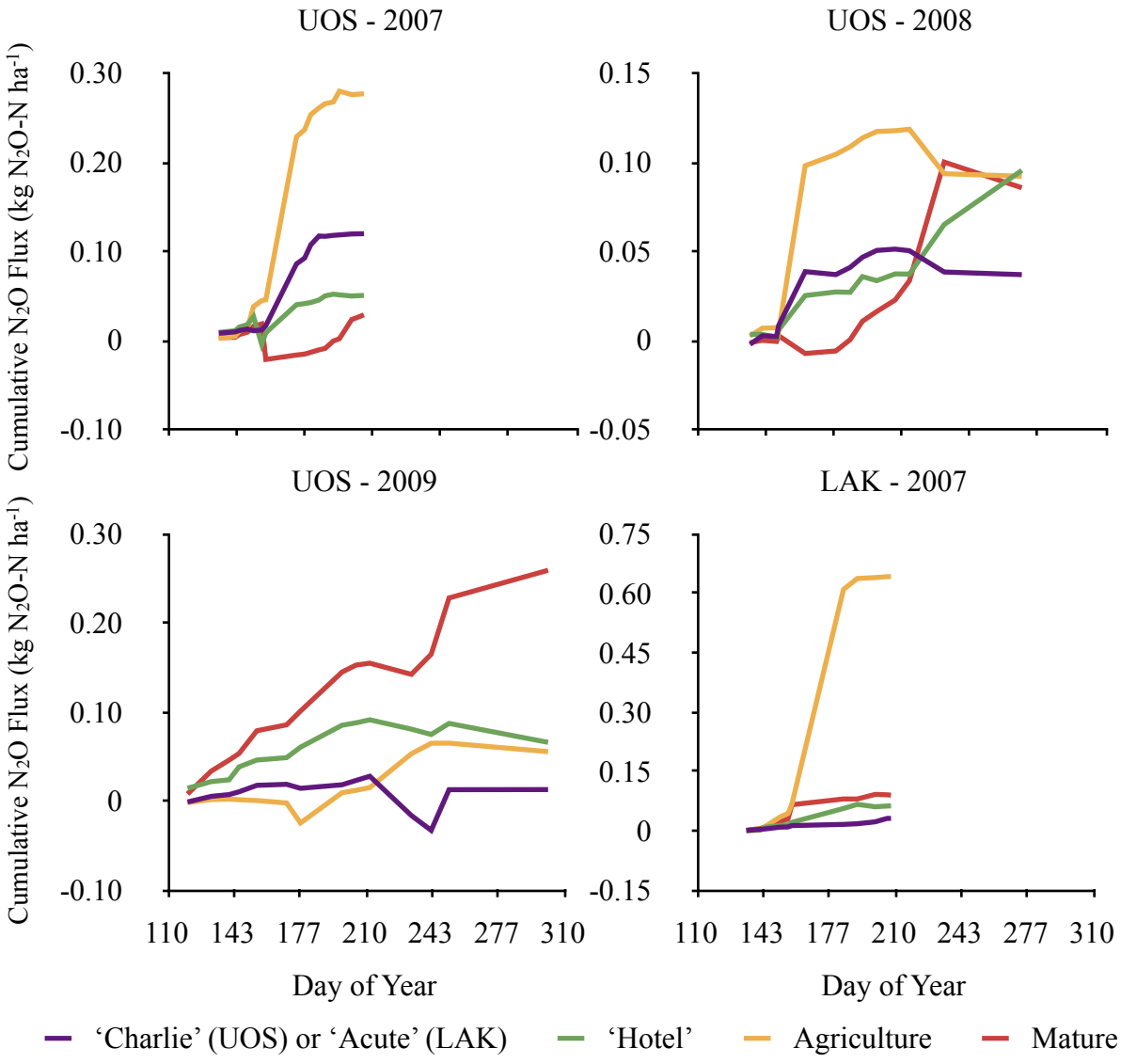

Figure C.1: Cumulative nitrous oxide fluxes (kg N_2O-N ha^{-1}) for two clones of willow ('Acute' or 'Charlie' and 'Hotel'), annual cropping (Agriculture) and mature trees (Mature).